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Cardiac Responses and Catecholamines Following Cervico-Thoracic Sympathectomy

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CARDIAC RESPONSES AND CATECHOLAMINES FOLLOWING CERVICO-THORACIC SYMPATHECTOMY

by

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BIOGRAPHY

Mary Ann Aberle Klouda was born in Peoria, Illinois, on January 8, 1937. In 1947 her family moved to California. She graduated from Notre Dame High School of San Jose, California, in June, 1954. In September of the same year, she enrolled at the College of Notre Dame in Belmont, California, where she pursued a course of study with a major in Chemistry. She was awarded the degree of Bachelor of Arts, cum laude, in 1958. In September of 1958 she enrolled at the Graduate School of Loyola University and began a program of study in the Department of Physiology. In November of 1959 she was awarded a National Institues of Health Predoctoral Fellowship, which she held until June of 1963. She is an associate member of the American Physiological Society and of the Society of the Sigma Xi.

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INTRODUCTION

It has been shown many times by various investigators that the result obtained from stimulation of the right sympathetic cardiac nerves is quite different from the result obtained when the left sympathetic cardiac nerves are stimulated. The effect most commonly observed with a right sympathetic stimulation is an acceleration of the heart rate. There is often also an augmentation in force of cardiac contraction. The principal effect observed with a left sympathetic stimulation is a great augmentation in force of cardiac contraction with only a slight acceleration of the heart rate.

Structurally and chemically, the right sympathetic cardiac nerves seem to be no different from the left sympathetic cardiac nerves. Why then should there be such a difference in the results obtained from stimulation of the two nerve supplies? The obvious answer seems to be that the terminations of the right and the left sympathetic nerves are not uniformly distributed throughout the heart. Instead, one area of the heart may receive its innervation primarily from the left sympathetic cardiac nerves while another area may receive its innervation primarily from the left sympathetic cardiac nerves.

It is widely accepted at the present time that norepinephrine is the actual chemical transmitter released at the endings of post-ganglionic sympathetic adrenergic nerves. Therefore, the concentration of norepinephrine in a tissue should give an indication of the relative concentration of adrenergic nerve endings in that tissue.

In the present study, cardiac tissue was analyzed for its content of epinephrine and norepinephrine using the fluorimetric method of analysis. It was found that a partially sympathectomized heart has a lower concentration of norepinephrine than does a normal heart. This result was to be expected since the norepinephrine present in the heart is presumably released from sympathetic nerve endings. Small samples of cardiac tissue were analyzed for their content of norepinephrine in an attempt to determine which areas of the heart are innervated by the right sympathetic cardiac nerves and which areas are innervated by the left sympathetic cardiac nerves.

CHAPTER I

REVIEW OF THE LITERATURE

A. Role of Epinephrine and Norepinephrine in the Mammalian Organism

The concept that norepinephrine is the neurohumoral transmitter released at the nerve endings of sympathetic adrenergic post-ganglionic nerves has only recently been elucidated. In 1904, after studying the adrenalectomized animal and comparing it with the sympathectomized animal, Elliot made the following statement, "Adrenaline might then be the chemical stimulant liberated on each occasion when the impluse arrives at the periphery." This statement is the first direct suggestion that sympathetic nerve activity might include the release of a highly active principle at the nerve endings, a "humoral transmission" (243). In 1905, Elliot extended his experimental research on the action of adrenaline and was able to show that the action of adrenaline on sympathetically innervated organs mimics the effect following excitation of the sympathetic nerves supplying that organ. For example, he found that adrenaline accelerated the heart as did the cardiac accelerator nerves. It constricted the blood vessels of the body unequally, but to much the same extent in the various organs as did excitation of the sympathetic nerves. Further he found that degeneration of the sympathetic nerves did not abolish the action of adrenaline. In fact, it seemed to increase the sensitivity of the organ to adrenaline. He also observed that neither the nerve cells, nerve fibers, nor the contractile muscle fibers could be irritated by

adrenaline. Therefore, he suggested that the stimulation takes place at the junction of the muscle and the nerve (67).

It was not until 1921 that Loewi provided the experimental evidence necessary to substantiate the theory proposed by Elliot concerning neurohumoral transmission. His experiments proved the existence of specific substances liberated from cardio-inhibitor as well as cardio-accelerator nerve fibers on stimulation. After these decisive findings a research was commenced which aimed at the identification of these neurohormones. Loewi's "Vagusstoff" was soon identified as acetylcholine. However the chemical nature of the sympathetic transmitter substance, Loewi's "Acceleransstoff", was not so easily determined.

In 1910, Barger and Dale published the results of an extensive study in which they reported a relationship between chemical structure and the "sympathomimetic" action of a great number of amines. Among 55 compounds tested, amino-ethanol-catechol (norepinephrine) exerted the strongest action on the blood pressure of a decerebrate cat. The authors stated that their results were compatible with the concept proposed by Elliot postulating the existence of a myoneural junction, the function of which is to receive the nerve impluse or the chemical stimulant substance. Further, they stated that this chemical substance liberated at sympathetic nerve endings produces effects which correspond very closely with the action of norepinephrine (22).

In 1933, Cannon and Rosenblueth postulated that there may be two types of sympathetic humoral agents, an excitatory one and an inhibitory one.

They theorized that there is probably only one substance released by the sympathetic nerve when it is stimulated. This substance they call substance M. However this substance can then unite in the effector cell with an excitatory substance, substance E, or an inhibitory substance, substance I. This combination results in the formation of the active "sympathin". Thus, a combination ME produces sympathin E, the excitatory sympathin, while a combination MI produces sympathin I, the inhibitory sympathin (45).

Stehle and Ellsworth, in 1937, reported the results of experiments in which they studied the effect of norepinephrine in decapitate cats. They found that the response agreed with that produced by stimulation of the hepatic nerves. They therefore suggested that norepinephrine may be the substance released when the hepatic sympathetic nerves are stimulated. However, they stated that their experiments did not show whether norepinephrine alone was effective or whether it reacted with a tissue substance to form sympathin E, which was then directly responsible for the effect (258). In 1938, Greer and co-workers, reported a close correlation between the response to norepinephrine and the response to the substance released by the liver following hepatic sympathetic nerve stimulation (124).

In 1946, von Euler published his observation on spleen extract. He found that extracts of fresh cattle spleen possessed pressor activity and that this extract closely resembled norepinephrine in its action. Biological tests, color reactions, and fluorescence reactions all indicated that the substance involved was norepinephrine (72). In the same year he published a study of a

sympathomimetic substance in extracts of mammalian heart, in which he concluded that the substance responsible for the sympathomimetic actions in the heart extracts is intimately related to some substance resembling norepinephrine (73). Von Euler also made an extensive study of nerve tissue extracts. He found that extracts of thoracic and lumbar sympathetic chains and the splenic nerves possessed a large pressor activity which was similar to norepinephrine. He also suggested that the norepinephrine in sympathetic nerves may be bound to lipids (74).

In 1947, von Euler and Schmiterlow showed that the normally occuring pressor substance in bovine and human blood resembles norepinephrine (101), and in 1948, Schmiterlow showed that the pressor activity in extracts of blood vessels behaved like norepinephrine. Through histological studies, he also showed a correlation between the amount of pressor substance and the amount of nervous structures present (243).

Bacq and Fischer studied the biological effects of various tissue extracts. In splenic extracts they found a high content of a pressor substance which seemed to be identical with norepinephrine. They also found a substance having the properties of norepinephrine in extracts from the sympathetic chain, splenic nerves, and stellate ganglion (21).

In 1948, von Euler analyzed extracts of splenic nerves using bioassay and colorimetric analysis. He found that the amount of l-norephinephrine in splenic nerves was 10-15 u gm per gram of nervous tissue. He also found 0.5 u gm of epinephrine per gram of nervous tissue (75).

As more and more evidence accumulated, the concept that norepinephrine may be the actual sympathetic neurotransmitter began to be accepted. The epinephrine and norepinephrine content of various tissues and organs was measured. Epinephrine-producing chromaffin cells were found in various organs of the body. All adrenergic nerves were found to contain large amounts of norepinephrine. However, very small amounts of epinephrine were also found in adrenergic nerves (76). Goodall, in 1950, reported the results of a colorimetric analysis of beef heart extracts. The auricles were found to contain 0.40 u gm of norepinephrine per gram of tissue and 0.04 u gm of epinephrine per gram of tissue. On the other hand, the ventricles contained 0.30 u gm norepinephrine per gram and 0.07 u gm epinephrine per gram (113).

Outschoorn, in 1952, studied the coronary sinus blood of dogs after stimulation of the cardiac sympathetic nerves and reported significant liberation of norepinephrine into the coronary blood. The plasma level of norepinephrine rose from a control level of 2.5 u gm per liter to a level of 7.5 to 10.0 u gm per liter after sympathetic stimulation (212).

Weil-Malherbe has shown that a large percentage of the epinephrine and norepinephrine occurring in plasma is associated with the blood platelets. His experiments demonstrated that when the plasma was centrifuged at 600 g, the platelets remained in suspension and the plasma content of epinephrine was 1.47 u gm per liter while the norepinephrine content was 4.25 u gm per liter. When the plasma was centrifuged at 3000 g, the platelets precipitated

and the epinephrine content of the plasma fell to 0.34 u gm per liter while the norepinephrine content fell to 1.20 u gm per liter (284). He also observed that, in heparinized blood, clumping of platelets occurs and results in low plasma levels of epinephrine and norepinephrine (287). However, Vendsalu, in his studies on epinephrine and norepinephrine in human plasma, was unable to find any difference between platelet-rich plasma (centrifuged at 150 g) and platelet-poor plasma (centrifuged at 16,000 g) (271).

In general, the amount of norepinephrine has been found to vary greatly from organ to organ, but it can be correlated with the extent of the sympathetic nerve supply to an organ. Accordingly, the spleen has been shown to be comparatively rich in norepinephrine, while the lung and striated muscle contain small amounts. In nerve-free placenta, no norepinephrine can be found. The norepinephrine content of various peripheral nerves also varies with the amount of post-ganglionic sympathetic nerve fibers in the nerve trunk. The concentration of norepinephrine in nerve endings is far higher than in the nerve trunk itself (79).

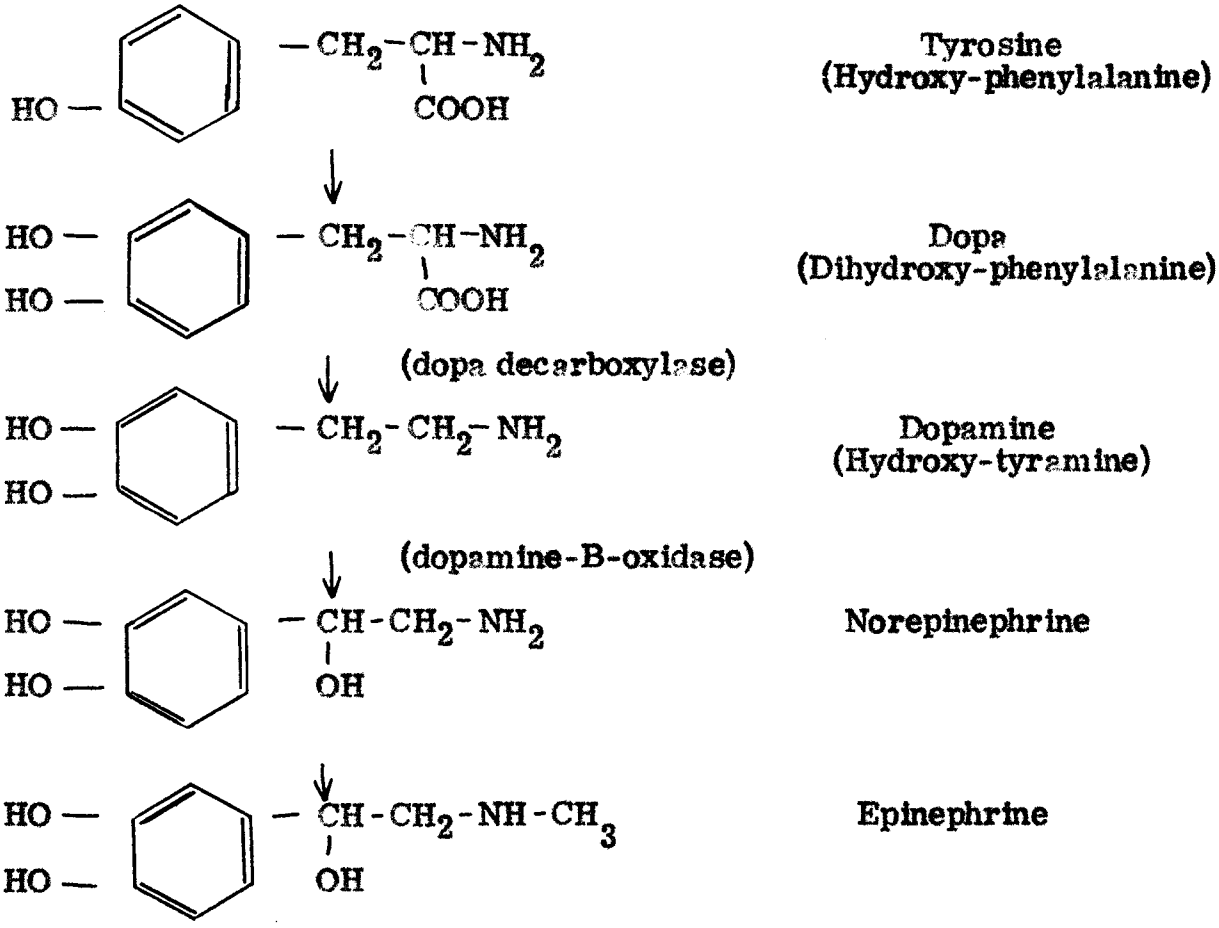
The brain also contains significant amounts of norepinephrine, the highest concentration being in the hypothalamus. The gray matter around the aqueduct, the tegmentum of the midbrain, the meduallary reticular formation, and the medial part of the thalamus also contain considerable amounts of norepinephrine. The remainder of the brain however contains very little norepinephrine. The histological site of formation and/or storage of norepinephrine in the brain is unknown; it may be situated in nerve cells, non-

medullated fibers, glia, or at several of these sites. Macroscopically, there is a high concentration of norepinephrine in all parts which contain the central representation of the sympathetic system. It has been shown that norepinephrine is lost from the hypothalamus whenever the sympathetic centers situated there are vigorously stimulated (273).

An analysis of the norepinephrine content of any tissue necessitates an extraction of the norepinephrine from the tissue followed by analysis of the tissue extract. Recently, however, Carlsson has developed a new histochemical method for visualization of norepinephrine in tissues. It involves the use of a fluorescence microscope and is many times more sensitive than previously available histochemical methods (48). Through utilization of this technique, it is conceivable that our knowledge of the distribution of norepinephrine in various tissues will be greatly enhanced.

B. Biosynthesis of Epinephrine and Norepinephrine

The pathway of formation of epinephrine and norepinephrine, as it is generally accepted today, is as follows:



Udenfriend, in 1956, showed that phenylalanine, tyrosine, and dopa could act as precursors of adrenal epinephrine and norepinephrine in the rat. This was the first direct evidence for the role of tyrosine and of dopa in the synthesis of epinephrine and norepinephrine (267). The intermediate between dopa and norepinephrine was postulated to be dopamine because the enzyme dopa decarboxylase had been found in the adrenal medulla.

Goodall, in 1957, reported on a study in which minced bovine or canine sympathetic nerves, sympathetic ganglia, or parasympathetic nerves were incubated with C-14 labeled tyrosine or C-14 labeled dopa. It was found that both bovine and canine sympathetic nerves and ganglia when incubated with either labeled tyrosine or labeled dopa, formed radioactive dopamine and norepinephrine. A very slight amount of radioactive epinephrine was also formed. Vagus nerves could form radioactive dopamine when incubated with labeled dopa but not when incubated with labeled tyrosine. No epinephrine or norepinephrine could be formed in either case (117). In a study on adrenal gland tissue, Goodall found that the formation of norepinephrine from dopamine required oxygen both in tissue slices and in homogenates. Adrenal slices formed epinephrine, norepinephrine, and dopamine from labeled tyrosine. Dopamine was suggested as the precursor to norepinephrine. Both dopa and dopamine could be used by the adrenal gland for the synthesis of norepinephrine and epinephrine (118). Therefore, Goodall proposed that the following pathway occurs both in the adrenal medulla and in sympathetic nerves and ganglia: Phenylalanine → tyrosine → dopa → dopamine → norepinephrine →

epinephrine (119).

The decarboxylation of dopa to dopamine by dopa decarboxylase appears to take place in the extragranular portion of the adrenal medullary cell. Recent evidence indicates that the chromaffin granules of the adrenal medulla are the actual site of norepinephrine synthesis from dopamine. The mechanism involved is not known. However, it would appear that the chromaffin granules thus serve not only as a storehouse for epinephrine and norepinephrine but are actually the site of norepinephrine formation from dopamine. If the norepinephrine is formed in the interior of the granules it would have to diffuse into the clear cytoplasm, there be methylated, and then re-enter the chromaffin granules (120). This sequence would be slow and may account for the slow restoration of the epinephrine content of the adrenal gland which many authors have observed following depletion.

The intracellular localization of epinephrine and norepinephrine has recently been the subject of many investigations. In studies on the perfused ox spleen, Eliasson showed that a change in the reaction of the perfusing fluid to the acid side using naturally occurring acids such as ascorbic, lactic, and citric acid caused a release of norepinephrine from the spleen. Extra release of norepinephrine could be observed even when the pH of the perfusate fell only from 6.9 to 6.6. Changes of this order could occur in vivo in conditions of hypoxia or reduced circulation. It had previously been shown by Hillarp that adrenal medullary granules lost their catecholamines during treatment with acids. Therefore Eliasson postulated that, if similar struc-

tures were present in sympathetic nerves, they may act in the same way. Nerve stimulation may cause a change in the membrane structures so that the contents of the granules are released (66). In an extension of the same study, von Euler showed that surface active agents such as detergents increased the release of norepinephrine from the perfused ox spleen. These same agents had been shown by Hillarp to release catecholamines from the granules of the chromaffin cells (102).

Norepinephrine is present not only in the axons of the nerve but also in the cell soma, as indicated by the fact that extracts of thoracic sympathetic ganglia contained approximately the same amounts of norepinephrine as did the fibers. It seems unlikely that the transmitter occurs free in the axonal tissue which contains amine oxidase and possibly other destructive enzymes. It was therefore suggested by von Euler and others that the transmitter is located in some specific structures within the axon rather than existing free in the axoplasm. They theorized that if norepinephrine were stored in such hypothetical structures, these might be distributed in a pattern similar to that actually found for norepinephrine. In this way the accumulation in the terminal parts could be satisfactorily explained.

Von Euler and Hillarp, in 1956, reported a study on homogenized spleen tissue and splenic nerves. They subjected the suspension to fractional centrifugation. A fine granular sediment was obtained which contained norepinephrine in a bound and protected form similar to that in the adrenal medulla. When this sediment was suspended in neutral isotonic saline, no norepineph-

rine activity could be detected. However if the pH was changed to 4 or lower, the norepinephrine went into solution. Heating or the addition of detergents also caused release of norepinephrine. It is postulated that these nerve granules, which store the transmitter, may also have the ability to synthesize norepinephrine. Thus, the chemical transmitter of the adrenergic nerves is believed to be stored intra-axonally in structural units which can be separated from the rest of the axonal tissue. If these structures also synthesize the transmitter, this synthesis must proceed at a high rate since adrenergic nerves are capable of releasing their neurotransmitter substance continuously on stimulation over a period of many hours. The rate of axoplasm flow is not sufficiently high to explain the rapid replenishment of the transmitter. On the other hand, it is possible that the granules are continuously moving from the pericaryon to the periphery. There is no reason to suppose that the granules leave the axon during stimulation; they probably only discharge their contents. Apparently the norepinephrine is well protected in the granules, since an organ like the spleen can be stored over a day at room temperature without any appreciable loss of norepinephrine. It has been shown that heart tissue can be stored at room temperature up to six hours before significant losses of norepinephrine occur (147).

The mechanism of release of the transmitter is unknown. It has been suggested that the amines are bound to proteins. The possibility of an intra-axonal ionic shift in connection with the nerve excitation volley, as a factor causing the necessary alteration in the binding capacity of the granules,

might be considered as a working hypothesis. Relatively high amounts of transmitter are found in the nerve trunk. The presence of the transmitter here seems to have no function. It probably has nothing to do with conduction. The intra-axonal structures containing the transmitter are probably present along the whole length of the neuron. Apparently they are accumulated at the endings, judging from the relative amounts of norepinephrine in the axon and in the organ. Since such granules have been demonstrated in homogenates of adrenergic nerves, it might be postulated that they are formed in the area around the nucleus and then transported to the periphery. It has been demonstrated that the axoplasm flow is not rapid enough to replenish the amounts of transmitter which are released at the periphery, but what it could do is replace the structural units which could then synthesize the transmitter at a high rate. When a post ganglionic adrenergic nerve fiber is cut and the distal end stimulated, there continues to be an appreciable amount of norepinephrine released from the nerve ending itself. The question arises whether this is due only to release of stored norepinephrine or whether the nerve ending is indeed synthesizing the transmitter (82).

Hillarp has recently made further investigations on the granules of the adrenal medulla. He has shown that the cytoplasmic granules are 0.1 to 0.6 u in diameter and have a high density. They have a membrane which is impermeable to proteins but permeable to small ions and molecules. They undergo lysis in hypotonic media with instantaneous release of catecholamines. These amines are normally bound within the granules in a non-diffusable state

Each ATP molecule probably holds four amine molecules by electrostatic forces. A protein component is probably also involved in the complex. This postulated storage complex breaks down immediately when the granule membrane is ruptured. During an in vivo stimulation, the amines and ATP are liberated from the storage complex without any release of the intragranular proteins (139).

C. Metabolism of Epinephrine and Norepinephrine in Various Organs

Little was known of the fate of norepinephrine and epinephrine in the body until recently. It is now generally accepted that norepinephrine and epinephrine are metabolized by a combination of O-methylation and oxidative deamination to yield the following major metabolites: 1) normetanephrine and metanephrine, the 3-methoxy analogues of the amines, 2) 3-methoxy - 4-hydroxy-mandelic acid, and 3) 3, 4-dihydroxy-mandelic acid.

Mono-amine oxidase was shown by Blaschko, in 1937, to cause an oxidative deamination of norepinephrine and epinephrine as well as many other amines. The enzyme is present in mammalian liver and various other organs. It can be demonstrated in all vertebrates and in many invertebrates (33). Many studies were carried out by various investigators in an attempt to determine whether mono-amine oxidase was the enzyme responsible for the inactivation of norepinephrine and epinephrine in vivo. An effort was made to apply to mono-amine oxidase at the adrenergic nerve endings a function similar to that of cholinesterase at cholinergic endings.

Schayer studied the metabolism of epinephrine containing isotopic carbon. He concluded that about 50% of administered epinephrine in the rat is inactivated by loss of methyl-amine, presumably through the action of amine oxidase (241). Burn investigated the effect of sympathetic denervation on the tissue content of mono-amine oxidase. In studies on the nictitating membrane, he found a fall in the amount of mono-amine oxidase after degeneration of the sympathetic fibers. The fall in enzyme content could be correlated with the

magnitude of the increased sensitivity of the denervated membrane to norepinephrine and epinephrine (41).

In studies on the perfused ox spleen, von Euler showed that amine oxidase inhibitors did not increase the release of norepinephrine from the perfused spleen. However they did increase the yield following nerve stimulation (90). In studies on liver tissue in vitro, it was shown that norepinephrine formed oxidation products indicating attack by mono-amine oxidase. However, the substance 3, 4-dihydroxy-norephedrine formed no such products indicating no attack by mono-amine oxidase. When a study of the urinary excretion of norepinephrine and of dihydroxy-norephedrine in man was made, it was found that 3.8% of the norepinephrine and 3.6% of the dihydroxy-norephedrine was excreted in the free form. Von Euler concluded that, since the dihydroxy-norephedrine was inactivated to the same extent as the norepinephrine although it is not attacked by mono-amine oxidase, some other enzyme system must play a role in the destruction of circulating catecholamines (90).

In 1957, Armstrong isolated 3-methoxy-4-hydroxy-mandelic acid from urine, Axelrod isolated normetanephrine and metanephrine from urine, and von Euler found 3-4-dihydroxymandelic acid in urine. And, in the same year, Axelrod reported the isolation from rat liver of an enzyme which catalyzes the O-methylation of norepinephrine and epinephrine. He called this enzyme catechol-O-methyl transferase (4).

These findings suggested three possible pathways for the metabolism of catecholamines: 1) oxidative deamination followed by O-methylation, 2) O-

methylation followed by oxidative deamination, or 3) both reactions occuring simultaneously. Many experiments were performed in an attempt to determine which pathway predominates in the in vivo metabolism of catecholamines. However, it may well be that the relative importance of each mechanism varies from tissue to tissue and from species to species. Through studies of urinary metabolites, Axelrod has shown that major pathway for metabolism of exogenous circulating norepinephrine and epinephrine is O-methylation followed by oxidative deamination. This was found to be the case in man and in the rat (7).

In vitro studies with both rat liver and rat brain showed that O-methylation was the predominant mechanism for metabolism of catecholamines in these tissues. A study on the distribution of catechol-O-methyl transferase in monkey tissue showed that the highest concentration is in the liver. High concentrations were also found in the brain, spleen, blood vessels, and peripheral nerves (6). It is probable that, in these tissues, O-methylation is indeed the most important metabolic pathway. Oxidative deamination by mono-amine oxidase may play only a minor role. It seems that the main function of mono-amine oxidase may be the oxidative deamination of normetanephrine and metanephrine. These two substances are actually better substrates for mono-amine oxidase than are norepinephrine and epinephrine.

In circulating blood and in the tissues mentioned above - liver, brain, spleen, blood vessels, and peripheral nerves - it has been clearly demonstrated that metabolism of catecholamines takes place principally by means

of O-methylation. However, in the case of other tissues (for example, the heart) this may not be true. In 1960, Goldstein published a comparison of metabolism of epinephrine and norepinephrine in the heart and in the liver. He found the catecholamines are to a large extent metabolized by mono-amine oxidase in the heart. The O-methyl transferase activity of the heart is much less than that of the liver. Goldstein concluded, therefore, that catecholamines in the heart are metabolized to a greater extent by mono-amine oxidase than by O-methyl transferase. In the liver, O-methyl transferase seems to be the enzyme responsible for inactivation of catecholamines (112).

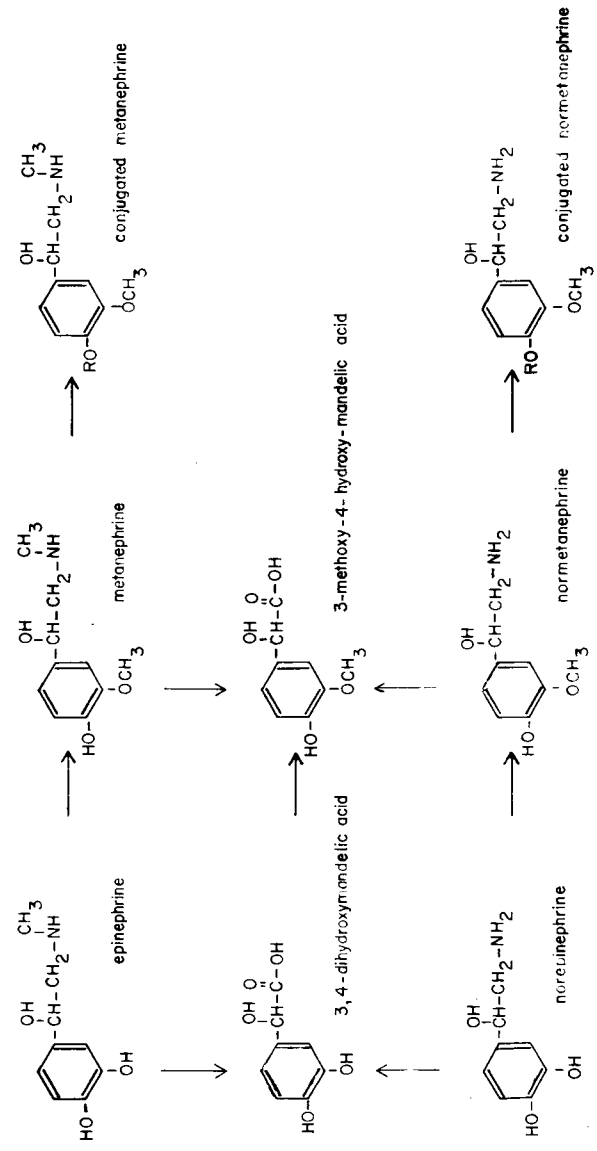
Crout, in 1961, showed that the mono-amine oxidase activity of the rat heart was five times the catechol-O-methyl transferase activity. Mono-amine oxidase inhibitors were shown to cause a significant increase in the norepinephrine level of the heart. A catechol-O-methyl transferase inhibitor had no effect on heart catecholamines. Injections of norepinephrine were given to the rats and the norepinephrine content of the heart and the circulating plasma were measured. In the presence of a mono-amine oxidase inhibitor, norepinephrine accumulated in the myocardium while clearance of norepinephrine from the circulating plasma was relatively unaffected. However, if catechol-O-methyl transferase were inhibited, the ability of the rats to metabolize circulating norepinephrine was severely impaired (61).

It seems, therefore, that any consideration of the metabolism of catecholamines must take into account the particular tissue involved and also the species concerned, since wide variations in metabolic pathways have been

demonstrated from one tissue to another and also between various species.

Figure 1

METABOLISM OF CATECHOLAMINES



D. Influence of the Sympathetic Nervous System on Cardiac Function

Hunt, in 1899, professed the view that the heart is under a continual influence of sympathetic impulses. This view of Hunt is today an accepted fact. Bronk was among the first to demonstrate the presence of continuous action potentials in the cardiac sympathetic nerves. He isolated one of the nerves running from the stellate ganglion and transected it. The proximal portion was then attached to a brush electrode, allowing the recording of efferent sympathetic impulses (36).

In 1945, Shipley and Gregg demonstrated that stimulation of the sympathetic cardiac nerves caused the heart to beat more vigorously, increased the cardiac output and the blood pressure, increased the coronary flow, and increased the coronary A-V oxygen difference. They postulated that the cardiac sympathetic nerves must liberate a cardiac stimulating substance (250).

Randall found that stimulation of the cardiac sympathetic nerves produced a large elevation in systolic blood pressure with a lesser elevation in diastolic pressure. Thus, a significant increase in pulse pressure occurred. The elevation of blood pressure was shown to be produced principally by an augmentation of ventricular contraction rather than by cardiac acceleration. (230). Randall has further demonstrated that accelerator fibers are more prominent in the right cardiac sympathetic nerves while augmentor fibers are more prominent in the left cardiac sympathetic nerves. These accelerator and augmentor fibers could not be functionally differentiated at any point

in their anatomical pathway from the spinal cord to the heart. The classification of a nerve fiber as accelerator or augmentor was believed to be determined by its site of termination in the heart (229).

In a study in which cardiometer recordings were analyzed, Kelso reported that sympathetic stimulation produced an increase in both the rate and the volume of ventricular systolic ejection. There was a smaller end-systolic volume with no significant change in the end-diastolic volume. By stimulation during isovolumetric conditions in the ventricles, it could be demonstrated that the augmentation depended primarily on changes in contraction of the myocardium rather than on alterations in either the peripheral resistance or the initial distension of the ventricles (162).

Sarnoff has shown that stellate ganglion stimulation augments the strength of atrial contraction and thus the atrial contribution to ventricular filling. The augmented atrial contraction was shown to take place in a shorter period of time. In addition, it was demonstrated that the power produced by the ventricle from any given mean atrial pressure was increased by stellate ganglion stimulation (237).

The importance of the sympathetic innervation to the heart in increasing the amount of work which may be done by the heart seems to be readily evident.

E. Sympathectomy and Possible Regeneration of Sympathetic Nerves

The sympathetic nervous supply of the heart has been studied in considerable detail. It is now generally agreed that the pre-ganglionic neurons have their cell bodies in the intermediolateral cell column of the spinal cord from the levels T1 through T5. These cells send fibers via the white rami of the upper five thoracic nerves to terminate in the cervical and upper five thoracic ganglia of the sympathetic trunk. The post-ganglionic neurons may have their cell bodies in any of the above mentioned sympathetic ganglia. The main pathway taken by post-ganglionic fibers to the heart is via the stellate ganglion, a combination of the inferior cervical ganglion and the first thoracic ganglion. Many supplementary pathways have been described, however, and it is possible that fibers from each of the cervical and upper five thoracic ganglia may reach the heart without passing through the stellate ganglion.

Gaskell, in the 1880's, reported several studies on the innervation of the heart in cold-blooded animals. He found that, when he stimulated the vagus nerve in the frog, he could produce either an acceleration or a slowing of the heart rate. However he later found that this nerve was actually a combination of the vagus and sympathetic nerves. By careful manipulation, the vagus and sympathetic components could be separated. Stimulation of the sympathetic before it combined with the vagus produced purely augmentor effects. Stimulation of the vagus before it combined with the sympathetic produced purely inhibitory effects. The effect of sympathetic stimulation in the frog was a marked acceleration and an augmentation of the contractions of

both auricles and of the ventricle (107).

Flack, in 1910, showed that the vagus and sympathetic nerves exert their effect on heart rate by way of the sino-auricular node. If the S-A node were frozen, the acceleration or the slowing of the heart rate could no longer be obtained (104). Woolard, in 1926, showed that the auricles and the A-V bundle are supplied by both parasympathetic fibers and sympathetic fibers while the ventricular muscle is supplied by sympathetic fibers only (294). He showed that, in the auricles, fibers from both systems appear to end intramuscularly. The fibers seem to spin a plexus around the muscle cell and ultimately enter the protoplasm of the muscle.

Kuntz showed that in man a discrete inferior cervical ganglion very rarely occurs. This ganglion is commonly fused with the first thoracic ganglion to form the stellate ganglion. Sometimes the second thoracic ganglion is also incorporated in the stellate ganglion. Kuntz reported a study of cadavers and still-born fetuses in which he found that cardiac sympathetic nerves may arise from the upper thoracic segments of the sympathetic trunk as well as from the cervical and stellate ganglia (179). White, in 1933, found the same situation to exist in the dog. He showed that the upper thoracic ganglia send fibers directly across the posterior mediastinum to the posterior cardiac plexus (292).

Kabat, in 1937, demonstrated that sympathetic cardio-accelerator fibers are present in the vagus nerve. When the vagus nerve was stimulated after treatment with atropine, cardiac acceleration was produced (154). Nonidez,

in 1939, reported a thorough study of the innervation of the heart. He showed that the superior cardiac nerve, which originates from the middle cervical ganglion, does not reach the heart, but ends on the walls of large arteries. The middle cardiac nerve carries most of the sympathetic supply to the heart. The inferior cardiac nerve was found to carry efferent fibers from the heart. Nonidez also observed that the vagus nerves carried sympathetic post-ganglionic fibers (209).

The precise site of termination of the motor fibers in the heart has not yet been clearly demonstrated. Nonidez questioned the existence of a nervous "terminal reticulum" in the heart, in which the vagus and sympathetic fibers merge into a syncytium of neurofibrils. Hillarp proposed that the innervation apparatus may consist of a nervous ground plexus, within which terminal axon ramifications are running. He theorized that each axon might innervate a certain number of cells which then react as a functional unit. This unit supposedly would not be innervated by one neuron alone, but by several neurons. By means of this overlap, the response of the autonomic effector system could be modified both by temporal and by spatial summation effects (135). Kuntz, however, pointed out that individual nerve fiber terminations have not been demonstrated to occur. He tended to favor the view that the autonomic neuroeffector formations are plexiform, anastomosing, neurofibrillar networks that lie in close proximity to the effector tissues (180). This problem remains unresolved even today.

Recently, Kisch has reported on some studies of human hearts. He

demonstrated that the muscle fibers of both the atria and the ventricles are less well supplied by nerve fibers than are the coronary vessels. However, by electron microscopy, fine nerve fibers could be found between muscle fibers even when no blood vessel was nearby. Axons with fine tapering extensions were found in contact with the sarcolemma of muscle fibers. Kisch cautions, however, that the question of whether a nerve in contact with the sarcolemma of a muscle is a centrifugal or a centripetal one cannot be decided by electron microscopy (169).

In a similar study on human hearts reported by Hirsch, it was observed that some large and small nerve trunks entered the myocardium directly, not in association with branches of the coronary arteries. These nerves, after they have pentrated the myocardium, have a scanty perineurium. The fine nerve fibrils in them appeared to extend directly into the contiguous myocardial tissues (141).

At the present time, therefore, our knowledge of the terminal innervation of the heart is very scanty. However, the function of the cardiac innervation can be studied in various ways. One of these is by denervation studies. In 1897, Hunt reported that removal of the right stellate ganglion in the dog or the cat caused a considerable slowing of the heart with little effect on the blood pressure. Removal of the left stellate ganglion had less effect on the heart rate and more effect on the blood pressure (149). This was one of the first demonstrations of a functional difference between the right and the left cardiac sympathetic nerves.

In 1926, Cannon reported experiments in which both the right and left vagal cardiac nerves were cut and both stellate ganglia were removed in the cat. The adrenal glands were also removed and the liver was denervated. A cardioacceleration was still observed when the cat changed from a resting to an active state. It was found that accessory accelerator fibers from the upper thoracic ganglia below the stellate were mediating the acceleration. When these were removed, the heart rate remained steady in spite of vigorous activity (43). In 1939, Cannon reported a study in which extracts of sympathetically denervated hearts were analyzed by bioassay. He could find no sympathomimetic activity in these extracts (44).

Raab, in 1947, reported a study in which a cardiac sympathectomy was performed in cats by removal of the caudal cervical and stellate ganglia along with the sympathetic trunk down to the eighth rib. The catecholamine content of the heart after sympathectomy as determined by colorimetric analysis was reduced 44% from the control level (223). Goodall studied the effect of cardiac sympathectomy in sheep. He performed four different types of operation. 1) Removal of the right superior and middle cervical ganglia caused an increase in the norepinephrine content of the heart. 2) Removal of the left superior and middle cervical ganglia had no effect on the norepinephrine content of the heart. 3) Removal of the right stellate ganglia decreased the norepinephrine content of the heart. 4) Removal of the left stellate ganglion had no effect. When all four operations were performed on the same animal, the norepinephrine content of the heart fell to the same level as it did when the right stellate

ganglion alone was removed. Goodall concluded that the right stellate ganglion is the ganglion of importance in controlling the norepinephrine content of the sheep heart (115). Goodall later continued this study on the sheep and also extended it to include the dog. By means of similar experiments, he showed that in the dog the right stellate and middle cervical ganglia seemed to be most responsible for control of norepinephrine in the heart. In the sheep the inferior cervical and upper thoracic ganglia on the right side seemed to have the greatest influence (116),

Many authors have observed regeneration of sympathetic fibers following a sympathectomy. In 1897, Langley reported a study in which the post-ganglionic fibers from the superior cervical ganglion were cut in the cat. The dilator muscles of the iris, the muscles of the orbit, and the erector muscles of the hairs were studied. Ten weeks after the operation, function started to return and by four months function was normal (182). Kilvington found that complete regeneration of vasoconstrictor fibers following section of the dog sciatic nerve required nearly seven months (163). Cannon observed that following section of cardiac nerves, function began to return at six weeks. He postulated that regeneration of cut cardiac nerves may occur in two months. Goodall observed that six weeks after a cardiac sympathectomy, the norepinephrine content of the heart began to rise and by four months it was at the normal level (116).

In 1957, Murray published a report of collateral sprouting in the sympathetic nervous system of the cat. Partial denervation of the superior cervical

ganglion in the cat was performed by severing one or more of the communicating rami from their respective thoracic nerves. Subsequently, nerve sprouts arose from the remaining intact pre-ganglionic fibers and formed synapses with ganglion cells which were denervated by the operation. This resulted in an almost complete functional recovery of the ganglion even when as many as 90% of the pre-ganglionic fibers had been divided. Four weeks after the operation, there was complete return of function. In a second series of experiments, the post-ganglionic fibers were divided. In the cat, the post-ganglionic trunk of the superior cervical ganglion is composed of two large and one or two smaller fascicles. The two large fascicles were divided, leaving only about 10% of the post-ganglionic trunk intact. The divided proximal and distal ends were ligated and separated by a distance of at least one cm. Ten weeks later, the function of the nictitating membrane had returned to normal. This seemed to be due to collateral sprouting of the remaining intact post-ganglionic fibers rather than to growth of axons from the site of division. In another experiment, the post-ganglionic trunk was completely divided and the proximal and distal ends ligated and separated as before. Stimulation of the cervical trunk ten weeks later produced no response of the nicititating membrane or the pupil (206).

Thus it seems that sympathetic nerve fibers do have a remarkable capacity for regeneration and for collateral sprouting.

CHAPTER II

METHODS

A. Surgical Procedures

The animals used in this study were mongrel dogs of both sexes. Control animals were anesthetized with Nembutal (30 mg/kg). The chest was rapidly opened by means of a mid-sternal incision and the heart removed as quickly as possible. The heart was washed free of blood and duplicate tissue samples were immediately taken from the following areas: 1) the right atrium, 2) the left atrium, 3) the right ventricular free wall, 4) the left ventricular free wall near the base of the heart, 5) the left ventricular free wall near the apex of the heart, and 6) the interventricular septum. The tissue samples were rapidly frozen by placing them on squares of aluminum foil lying on slabs of dry ice. The twelve samples from each heart were then kept in the frozen state until time of the chemical analysis. The average size of the atrial samples was approximately 0.5 gms while the average size of the ventricular samples was approximately 1.6 gms.

Sham operated animals were prepared and their hearts compared with those of control animals. The animals were anesthetized with Nembutal (30 mg/kg) and prepared for sterile surgery. A skin incision was made over the second rib on either the right or the left side. The muscles were severed, the second rib was stripped free of its periosteum, and the rib itself was excised. The surgery was terminated at this point. The muscles were

sutured together and the skin incision was closed. Three to four days later these animals were sacrificed in the same manner as described for the controls.

The majority of the animals in this study underwent a unilateral cervico-thoracic sympathectomy. The surgery was performed in the same manner as described for the sham operated animals. Following removal of the second rib, the pleura was pierced, allowing an excellent view of the stellate ganglion. This ganglion was removed along with the thoracic sympathetic trunk down to the T5 ganglion inclusive. The caudal cervical ganglion and both the ventral and dorsal ansa subclavia were likewise removed. As an added precaution, the vagus nerve was severed and approximately a one inch segment removed in the area of the caudal cervical ganglion. This was done in order to interrupt any sympathetic nerves whose pathway to the heart might be by way of cardiac nerves traveling with the vagus nerve. Upon completion of the surgery, the pleura was sutured together and the chest was closed in the manner described above.

Other animals underwent a bilateral cervico-thoracic sympathectomy. The procedure described above was performed on first one side and then the other. There was a one to two week interval between the two stages of the operation. In order to avoid the problems involved in maintaining a bilaterally vagotomized animal the vagus nerve was not cut in the second stage of the operation.

The animals were sacrificed at various periods of time after the sympa-

thectomies were performed and cardiac tissue samples were taken in the manner described previously. On approximately one-half of the unilaterally sympathectomized animals, a functional study was made before sacrifice of the animal. For these experiments the animals were anesthetized with α-Chloralose (90 mg/kg) and the chest was opened by means of a mid-sternal incision. A unipolar electrode was positioned on the remaining intact stellate ganglion and all four chambers of the heart were cannulated. Pressures were recorded from the four chambers by means of Statham P23Db transducers whose output was fed into a Grass polygraph recorder. A Grass Model S5 stimulator was used to stimulate the stellate ganglion. The stimulus usually employed had a duration of 5 msec., a frequency of 10 per second, and an intensity of 5 volts. After the recordings of the functional response to stellate stimulation following unilateral sympathectomy were obtained, the heart was removed by quickly severing all connecting vessels. It was immediately washed free of blood and cardiac tissue samples taken as previously described. A time period of approximately fifteen to twenty minutes was allowed to lapse between the last stellate stimulation and the removal of the heart to allow time for metabolism of any newly released norepinephrine.

B. Chemical Analysis of Cardiac Tissue

All cardiac tissue samples were analyzed for their content of epinephrine and norepinephrine according to the following procedure. The frozen tissue sample was weighed on a Roller-Smith balance. It was then chopped into small pieces and placed in a conically shaped homogenizing tube together with 5 ml. of 0.4N perchloric acid. The tube was then immersed in a container of ice water in order to keep the tissue cold during homogenization. A glass pestle attached to a Sargent cone drive stirring motor was inserted into the tube. The tissue was extracted once and the homogenate poured into a centrifuge tube placed in ice water. In some cases it was necessary to repeat the extraction with a second 5 ml. of 0.4N perchloric acid. After the tissue had been completely homogenized, the conical tube and the glass pestle were rinsed with 1 to 2 ml. of 0.4N perchloric acid which was then added to the combined extracts. The homogenate was centrifuged at 4° C. and 3000 rpm (1500 g) for 15 minutes. The supernatant was then poured off and frozen overnight.

After the tissue extract had thawed the pH was adjusted to approximately 4.5 by dropwise addition of 5N potassium carbonate solution. A Beckman pH Meter, Model G, was used to check the pH at this point. The potassium perchlorate formed was then spun down by centrifugation at 4° C. and 3000 rpm (1500 g) for 5 minutes. The volume of the supernatant at this point was 8 to 15 ml. To the supernatant was added 10 ml. of 0.2N sodium acetate solution (pH 8.5) and 4 drops of 0.05% cresol red indicator. The pH of this solution

was adjusted to approximately 8.3 by dropwise addition of 0.5N sodium hydroxide solution. The extract was then added to a previously prepared alumina column.

The columns consisted of glass tubes 35 cm. in height having an internal diameter of 12 mm. which were constricted at one end to an opening 4 to 5 mm. in diameter. A glass wool plug had been placed in the narrow opening of the tube and one gram of alumina was placed into the tube. This alumina was then repeatedly washed with 0.2N sodium acetate solution until the pH of the alumina column was approximately 8.5. Thymol blue indicator was added to the sodium acetate solution being poured through the columns in order to check the pH of the washings. To this prepared alumina column was added the tissue extract which had been brought to pH 8.3.

After the extract had run through the column, the alumina was washed by addition of 10 ml. of 0.2N sodium acetate solution to the column. The filtrates were discarded. The catecholamines were subsequently eluted from the alumina column by addition of 10 ml. of 0.25N acetic acid to the column. The eluate was collected in a test tube and was now ready to be chemically analyzed for its content of epinephrine and norepinephrine.

The eluate was mixed with a stirring rod in order to ensure a uniform distribution of the catecholamines in the eluting solution. A 1 ml. aliquot of the eluate was pipetted into each of five 10 ml. volumetric flasks. The first two aliquots were carried through the fluorescence reaction. The second two aliquots served as blanks. The fifth aliquot was used in adjusting the pH of

the solution.

The fifth aliquot was treated as follows: 1 ml. of a pH 6.0 acetate buffer and 2 drops of 0.1% brom cresol purple were added to the flask containing the extract. The pH was then adjusted to approximately 6.2 by dropwise addition of 0.5N sodium hydroxide solution. The amount of sodium hydroxide added was noted. Nothing further was done with this aliquot.

The first two aliquots were then treated as follows: 1 ml. of a pH 6.0 acetate buffer was added. Then the amount of 0.5N sodium hydroxide solution necessary to bring the pH to approximately 6.2 was added. Next 0.1 ml. of 0.25% potassium ferricyanide solution was added as an oxidizing agent. The flasks were stoppered and inverted to mix the contents and the oxidation was then allowed to proceed for five minutes. At the end of this time, 1 ml. of a freshly prepared solution of 9 parts 5N sodium hydroxide solution and 1 part 2% ascorbic acid solution was added. The flasks were again inverted to mix the contents and a period of three minutes allowed for development of fluorescence. At the end of this time the solution was diluted to 10 ml. with triple distilled water, thoroughly mixed by inverting the flask several times, poured into a glass cuvette, and the fluorescence read.

The third and fourth aliquots were merely diluted to 10 ml. with triple distilled water and the fluorescence read in order to determine how much fluorescence was contributed by the cuvette, the water, and the eluate itself.

MATERIALS

1) Perchloric Acid, 70 - 72%, A. C. S., J. T. Baker Chemical Company

2) Potassium Carbonate, Crystal, A. C. S., J. T. Baker Chemical Company

3) Pyrex Wool Filtering Fiber, Owens-Corning Fiberglas Corporation

4) Alumina, Adsorption, 90 - 200 Mesh, Fisher Scientific Company

5) Sodium Acetate, Granular, Analytical Reagent, Mallinckrodt Chemical Works

6) Cresol Red, Sodium Salt, Allied Chemical Corporation

7) Thymol Blue, Sodium Salt, Allied Chemical Corporation

8) Sodium Hydroxide, Pellets, A. C. S., J. T. Baker Chemical Company

9) Glacial Acetic Acid, Reagent, E. I. Dupont De Nemours and Company

10) Brom Cresol Purple, Water Soluble, Chicago Apparatus Company

11) Potassium Ferricyanide, Crystal, A. C. S., J. T. Baker Chemical Company

12) l-Ascorbic Acid, Eastman Kodak Company

C. Discussion of Chemical Analysis

The first step in the chemical analysis of the content of epinephrine and norepinephrine in a tissue is the preparation of the tissue extract. The three agents most commonly used for extraction are 1) acid ethanol, 2) trichloracetic acid, and 3) perchloric acid. When acid ethanol is used as the extracting agent, the ethanol must be removed by evaporation at low temperature and low pressure. This often causes loss of epinephrine and norepinephrine. When trichloracetic acid is used as the extracting agent, it can be removed by extraction with diethyl ether. This also can cause appreciable loss of epinephrine and norepinephrine. Perchloric acid seems to be the ideal extraction fluid. The acid can be neutralized with potassium carbonate. This leads to the formation of potassium perchlorate, which is only slightly soluble in cold water. Most of the perchlorate is thus precipitated. The method used in this study for the preparation of the tissue extracts is that described by Bertler et. al. (27). All steps of the extraction were carried out at a temperature near 0° C. In fresh tissue extracts, a fine suspension is often present. The extract was placed in a freezer overnight, according to the method of Bertler. The following day this suspension could be easily spun down together with the potassium perchlorate after thawing and neutralization.

The next step in the chemical analysis is purification of the crude extract. The method of purification by adsorption on aluminum oxide was developed by Lund (191). For adsorption, Lund found that 1 gm. of aluminum oxide was

suitable for solutions containing up to a few micrograms of catecholamines. Lund also noted that the optimum pH for adsorption of catecholamines on aluminum oxide is 8. 4. By far the great majority of workers in the field of catecholamine analysis have used the method of adsorption on aluminum oxide as the means of purifying their tissue extract or plasma sample. Most workers are very careful to bring their sample to approximately pH 8. 4 before adding it to the alumina. However a great many workers make no attempt to bring the alumina itself to pH 8. 4. The alumina as it is purchased from the manufacturer is usually highly alkaline. Addition of the sample to this alumina without checking its pH could lead to partial destruction of the catecholamines in the sample. Some workers recommend acid washing the alumina. If this acid washed alumina is then used without checking its pH, incomplete adsorption of the catecholamines could result if the pH of the alumina is below 8. 4. So it seems that, whether the alkaline alumina or the acid washed alumina is used, adjusting the alumina to the optimum pH is essential for complete adsorption of catecholamines.

In this study, the alumina was not acid washed. One gram of alkaline alumina was added to a glass column containing a glass wool plug. About 10 ml. of 0. 2N sodium acetate solution containing thymol blue as an indicator was then added to the alumina in the column. Following the recommendation of Weil-Malherbe (281), a glass rod was gently worked up and down in the alumina column to remove any air bubbles and to prevent the alumina from clumping. At no time was the alumina column allowed to run dry, since

this may lead to the formation of air channels through the alumina column. The column was repeatedly washed with sodium acetate solution containing thymol blue until the pH of the filtrate was 3.5 as indicated by the color.

The tissue extract was adjusted to pH 8.3 in the manner described in the previous section of this chapter. After adsorption the column was washed with sodium acetate solution. At no time was the alumina column washed with any thing other than pH 8.5 sodium acetate solution. Some workers recommend that the washing with sodium acetate solution be followed by a washing with distilled water. This recommendation was not followed for fear that some of the catecholamine might be lost if the pH of the alumina were to change from 8.5.

Pyrex glass wool was used as a plug according to the recommendation of Ludemann (187). He found that filter paper or cotton was unsuitable since, even after repeated washings, these substances tended to provide traces of fluorescence. A sintered glass filter has been recommended by some workers and was tried in this study but it was found that the alumina particles tend to clog the filter. Therefore a pyrex glass wool plug seems to provide the best filter.

The elution of the catecholamines from the alumina column was carried out by the addition of 10 ml. of 0.25N acetic acid to the column as recommended by von Euler (98). The eluate was then ready for the third step in the chemical analysis, the formation of the fluorescent products.

There are basically two types of chemical reactions by which epinephrine

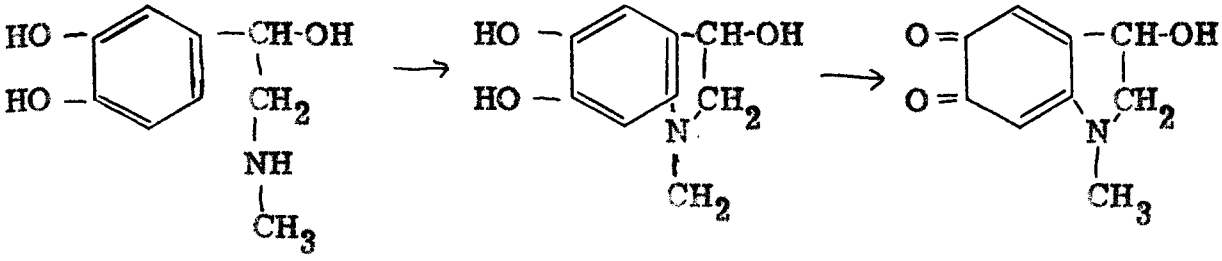
and norepinephrine can be made to yield fluorescent products. One analysis involves the formation of tri-hydroxy-indole compounds while the other analysis involves the formation of ethylene diamine condensation products. The ethylene diamine method is performed in the following manner (281). To the eluate from the alumina column is added ethylene diamine. This mixture is then heated at 50° C. for 20 minutes, cooled to room temperature, and saturated with solid sodium cholride. The solution is extracted with isobutanol, the isobutanol layer removed, and its fluorescence read. The condensation reaction takes place in alkaline solution. It is immediately preceded by an oxidation of the epinephrine and norepinephrine to their corresponding oxidation products. According to the authors, the most notable advantage of this method is that the unstable and highly reactive oxidation products are trapped in their nascent state and quantitatively converted to completely stable condensation products. The ethylene diamine condensation method is less specific than the tri-hydroxy-indole method. Several catechol derivatives other than epinephrine and norepinephrine will form condensation products with ethylene diamine but will not carry out indole ring closure.

The tri-hydroxy-indole method of analysis of epinephrine and norepinephrine was developed by Lund (190). It involves basically the oxidation of epinephrine and norepinephrine, followed by the addition of strong alkali to produce the fluorescent products. The yellow green fluorescence which appears in solutions of epinephrine after addition of strong alkali was studied by Paget in 1930. Gaddum and Schild in 1934 found that the reaction required

oxygen. In 1948 the studies of Ehrlen led him to conclude that the formation of the fluorescent compound was due to a rearrangement of the oxidation product. Ehrlen gave the correct formula for the fluorescent compound of epinephrine as 1-methyl-3, 5, 6-tri-hydroxy-indole. Attempts to utilize this method for the quantitative estimation of catecholamines were largely unsuccessful because of the instability of the fluorescent compounds. Lund demonstrated by oxygen titration that two atoms of oxygen per mole of epinephrine are required to produce the fluorescing molecule. In excess oxygen the fluorescence quickly reaches a maximum and then rapidly disappears. Thus the destruction of the fluorescent molecule also involves an oxidation breakdown (131). Ehrlen modified the chemical analysis by the addition of a reducing agent by which further oxidation of the fluorescent compound was prevented. In his analysis Ehrlen used potassium ferricyanide as the oxidizing agent. Lund adapted the method of Ehrlen and used manganese dioxide as the oxidizing agent. Lund in 1950 was the first to differentially determine epinephrine and norepinephrine using the fact that the oxidation of norepinephrine at low pH's proceeds very slowly while epinephrine is still rapidly oxidized (191). All later methods for the determination of epinephrine and norepinephrine involving the formation of tri-hydroxy-indole compounds are modifications of the method described by Lund in 1950.

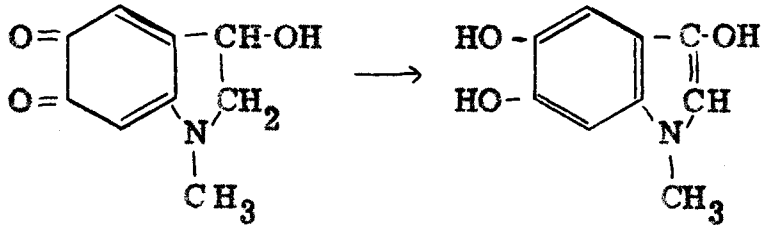
The names given to the oxidation products of epinephrine and norepinephrine are adrenochrome and noradrenochrome, respectively, while the fluorescent products are named adrenolutine and noradrenolutine. The

oxidation reaction for epinephrine is as follows:



Epinephrine Adrenochrome

The addition of strong alkali results in the following rearrangement:



Adrenochrome Adrenolutine

The procedure used by Lund is as follows: the catecholamines are adsorbed on aluminum oxide at pH 8.5. After elution with 0.2N acetic acid, the pH of the solution is about 4. Lund states that the difference in oxidizability between epinephrine and norepinephrine permits the determination of these two substances in the same sample. The epinephrine is oxidized quantitatively to adrenochrome within the entire pH range of 3 to 7. Only 5% of the norepinephrine is oxidized to noradrenochrome at pH 3.0 and quantitative oxidation at pH 6.5. Therefore, one sample of eluate is brought to pH 3.0 and oxidized. A second sample of eluate is brought to pH 6.5 and oxidized. After rearrangement with strong alkali to the corresponding lutines and

stabilization with ascorbic acid, the fluorescence intensity can be measured. The difference between the fluorescence in the samples oxidized at pH 6.5 and at pH 3.0 makes possible a calculation of the amount of epinephrine and the amount of norepinephrine in the eluate.

Von Euler and Floding, in 1955, published a modification of the method of Lund (85). Potassium ferricyanide was used as the oxidizing agent rather than the manganese dioxide used by Lund. The oxidation was carried out at pH 6.0 and at pH 3.5. The oxidation of norepinephrine at pH 3.5 is very slow. However, the author noted that the oxidation of epinephrine at this pH is also slow but could be enhanced by the addition of zinc sulfate.

The method used in the present study for the chemical analysis of epinephrine and norepinephrine is based on the method described by von Euler and Floding in 1955. However, a great many modifications have been introduced. Some of these have been on the advice of other investigators in this field and some have been a result of the personal experience of this writer. Therefore, the chemical analysis will now be described in detail.

The differential determination of epinephrine and norepinephrine by oxidation at two pH's was checked. It was found that, in the absence of zinc sulfate, the fluorescence of a standard norepinephrine solution oxidized at pH 3.5 was approximately 5% of the fluorescence of the same solution oxidized at pH 6.0. The fluorescence of an epinephrine solution oxidized at pH 3.5 in the absence of zinc sulfate was approximately 50% of the fluorescence of the same solution oxidized at pH 6.0. By the addition of zinc sulfate as a

catalyst and lengthening of the period of time allowed for oxidation, it was possible to increase the fluorescence of an epinephrine solution oxidized at pH 3.5 so that it equalled the fluorescence of the same solution oxidized at pH 6.0. However, under identical conditions, the fluorescence of norepinephrine solution oxidized at pH 3.5 was also increased to approximately 75% of the fluorescence of the same solution oxidized at pH 6.0.

In a report published by von Euler and Floding in 1956 (87), the authors recommend the addition of ethylene diamine tetra-acetic acid (EDTA) to the sample. They state, "It has been found essential for the differential estimation of adrenaline and noradrenaline to add this substance. It has no apparent influence on the results of chemical estimation of the total catechols, but its presence prevents the often occurring oxidation of noradrenaline at pH 3.5, which gives too high apparent adrenaline figures". Von Euler also points out that when EDTA is added to the sample, the amount of zinc sulfate must be increased 2 to 4 times to ensure quantitative oxidation of epinephrine at pH 3.5. It was found by this writer that the addition of EDTA did indeed decrease the fluorescence of a norepinephrine solution oxidized at pH 3.5. However, it also decreased the fluorescence of an epinephrine solution oxidized at pH 3.5. No suitable combination of amounts of zinc sulfate and EDTA was found which would allow 100% oxidation of epinephrine at pH 3.5 and only 5% oxidation of norepinephrine. Therefore it was decided to carry out the oxidation reaction only at the higher pH and to report the results as total epinephrine plus norepinephrine.

According to von Euler, treatment with potassium ferricyanide for 2 to 5 minutes at approximately pH 6 gives maximal fluorimetric readings for both epinephrine and norepinephrine (85). The pH should be from 6.0 to 6.5. Therefore in this study the sample was titrated to approximately pH 6.2 and the oxidation was carried out with potassium ferricyanide for 5 minutes. The oxidation was stopped by the addition of ascorbic acid and sodium hydroxide solution. The ascorbic acid and the sodium hydroxide solution must be added simultaneously. If the alkali were to be added first, the fluorescent lutines formed would be oxidized by the potassium ferricyanide. If the ascorbic acid were to be added first, some of the oxidation product may be reduced and thus not be converted to the fluorescent lutines when the alkali is added. Not less than 1 mg. of ascorbic acid must be present in 10 ml. of alkaline lutine solution in order to prevent oxidation of the lutines. However, if more than 4 mg. of ascorbic acid are present per 10 ml. of solution, the intensity of the fluorescence will decrease in proportion to the increasing amounts of ascorbic acid (190). In the present study, 2 mg. of ascorbic acid were always present in the final 10 ml. of lutine solution.

The fluorescence of the alkaline lutine solutions remains constant for 1 hour (190). Price and Price (218) have introduced a modification at this point of the analysis. Before reading the fluorescence of the lutine solutions, they convert the pH from about pH 13 to approximately pH 5 by the addition of 5N acetic acid. This is done in order to reduce the fluorescence of dopa and dopamine. These two substances will be adsorbed on the alumina in the

same manner as epinephrine and norepinephrine. Therefore they will be present in the eluate in the same amount in which they occurred in the original sample. However, with the standard tri-hydroxy-indole analysis, the fluorescence produced by these compounds is very small. For example, the fluorescence intensity of dopamine is less than 1% of that of epinephrine in equimolar solutions. A special modification of the tri-hydroxy-indole analysis is necessary in order to determine these substances (49). Therefore, there does not seem to be much advantage to acidifying the lutine solution in order to reduce the fluorescence contributed by dopa and dopamine. In addition, a disadvantage is that the lutines are unstable at pH 5, so that all readings of fluorescence must be made at exactly the same time after addition of the alkali in order that the percentage of destruction which has taken place will be the same for every sample. In the present study the fluorescence of the lutine solutions was read at the alkaline pH.

In the present study, the sample blanks consisted of 1 ml. of eluate plus 9 ml. of triple distilled water. The fluorescence of this sample blank was then subtracted from the fluorescence of the sample. Various authors have developed different ways of producing blanks. In the method of Lund, the blank is treated in exactly the same way as the sample except that the ascorbic acid is omitted from the sodium hydroxide solution used to produce the fluorescent product. The fluorescent lutines formed are therefore oxidized and the fluorescence of the lutine solution gradually fades. These are known as "faded blanks". The fluorescence of the blanks is not read until 30 minutes

after the addition of the alkali in order to allow time for the fluorescence to fade. In the original method of von Euler (85), faded blanks are also used. Again ascorbic acid is omitted from the analysis. The blank is read 2 minutes after the addition of the alkali. Von Euler states that in the presence of ferricyanide the fading of the lutines is much more rapid than with manganese dioxide as used in the method of Lund.

In a later publication, von Euler (87) reports a different way of producing blanks. All the reagents are added to the sample with the exception of the ferricyanide. Thus no new adrenochrome or noradrenochrome will be formed, but any adrenochrome or noradrenochrome already present in the sample will be rearranged and stabilized as the fluorescent lutines. Price and Price slightly modified this method of producing blanks (218). They add the ascorbic acid to the sample first, followed by the ferricyanide and then the sodium hydroxide. In this technique, all constituents of the final mixture are present equally in samples and blanks. The oxidation of epinephrine and norepinephrine is prevented by the addition of ascorbic acid prior to the addition of ferricyanide. Consequently fluorescence does not develop when the mixture is made alkaline.

Faded blanks tend to give rather erratic and non-reproductible fluorescence readings. Blanks formed by omitting the potassium ferricyanide or by adding the ascorbic acid before the ferricyanide are essentially identical. Both methods measure the fluorescence due to any adrenochrome or noradrenochrome present in the original sample. In this study, when samples

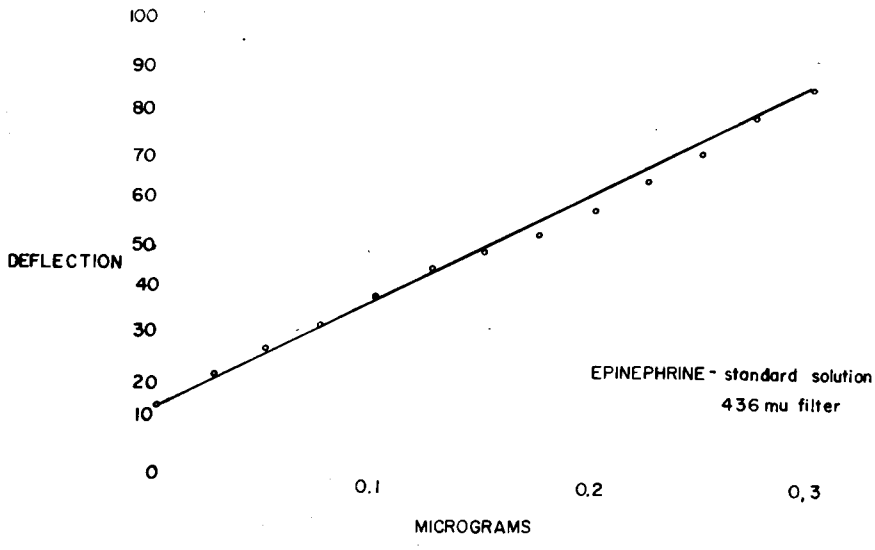
of eluate were analyzed as blanks by addition of the ascorbic acid before the potassium ferricyanide, the blanks always tended to read high. If a freshly made solution of epinephrine or norepinephrine were analyzed in this manner, the blanks consistently read low. In fact the readings were not significantly higher than the reading of the triple distilled water alone. However if the solution of epinephrine or norepinephrine were several hours old, the blanks tended to read high. This was most likely due to the fact that some oxidation had taken place and there was some adrenochrome or noradrenochrome present in the original solution. This is probably also the explanation for the high reading obtained when samples of the eluate are analyzed as blanks in this manner.

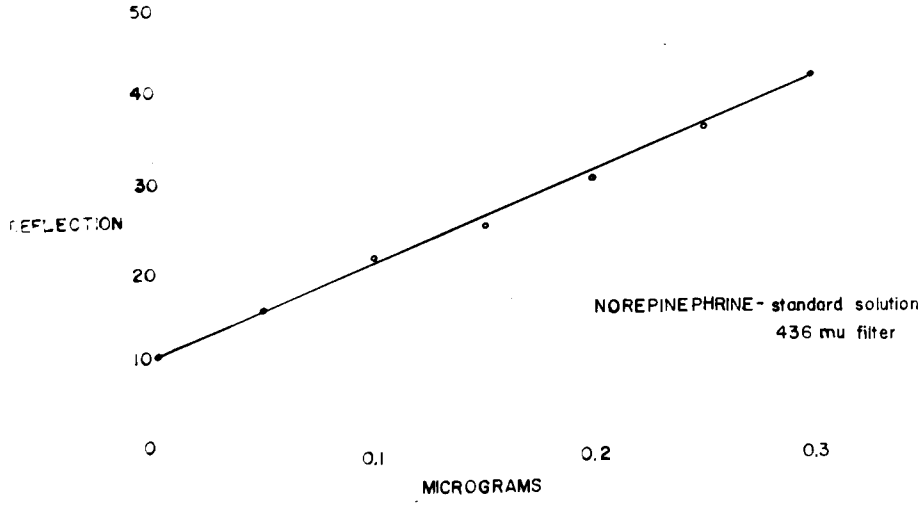
There is ample opportunity for some oxidation to occur during theprocess of extraction of the catecholamines from the tissue sample and the subsequent purification of this extract before it is ready for chemical analysis. If the blank is treated in the way described above, the reading will be high since a small amount of the catecholamines originally present in the tissue sample will already have been oxidized to adrenochrome and noradrenochrome. If this blank reading is subtracted from the sample reading, the resulting fluorescence will not give a true indication of the amount of catecholamines present in the original tissue sample. A reagent blank containing all of the reagents but no sample has essentially the same fluorescence as the triple distilled water alone. This indicates that the reagents do not contribute any fluorescence of themselves. It seems there that a valid sample blank

may be produced by merely diluting a sample of the eluate with triple distilled water. This will give a value for the amount of fluorescence contributed by the eluate, the water, and the cuvette. When this reading is subtracted from the sample reading, the resulting fluorescence is due to the epinephrine and norepinephrine present in the original tissue sample. This is the way the blanks were produced in the present study.

Figure 2

CONCENTRATION-FLUORESCENCE CURVES





The effect of indicators on the final fluorescence of the lutine solution was studied. It was found that every indicator tested tended to have a quenching effect on the fluorescence of the lutine solution. Therefore, precautions were taken to ensure that no indicator was present in the final solution whose fluorescence was to be read. The alumina columns were washed with sodium acetate solution containing thymol blue indicator. After the washing, traces of this indicator remained on the alumina. When added to the alumina column, the sample contained cresol red indicator. Traces of this indicator also remained on the alumina column. However, the column was then washed with clear sodium acetate solution which removed all indicator from the alumina. When the sample was eluted with acetic acid, the resulting eluate was clear. In the adjustment of the pH prior to oxidation, no indicator was added to the aliquot of eluate to be oxidized. Rather a separate aliquot was used which was titrated to the proper pH in the presence of an indicator and then discarded.

Standard solutions of epinephrine and norepinephrine were analyzed in the same manner as the tissue extract. The pH of the sample was adjusted to 8.3 and the sample was then added to a previously prepared alumina column. Following elution with acetic acid, the eluate was analyzed in the manner described previously. These standard solutions were freshly made immediately before use and were stable for only a few hours. Standard solutions contained 0.10 u gm. (as free base) of epinephrine or norepinephrine per ml. in 0.1 N acetic acid. They were prepared from a stock solution

which contained 500 u gm. (as free base) of epinephrine or norepinephrine per ml. in 0.1 N hydrochloric acid. The stock solutions were kept refrigerated and were stable for several months. They were prepared from dry l-epinephrine bitartrate and l-norepinephrine bitartrate purchased from Sigma Chemical Company.

Recovery studies of epinephrine and norepinephrine were made in two ways. In the first study, standard solutions of epinephrine and norepinephrine were adsorbed on alumina, eluted, and analyzed. The readings obtained were compared with those of standard solutions of epinephrine and norepinephrine which were analyzed without prior adsorption an alumina. The average recovery of epinephrine in these experiments was 95% with a range from 89 to 99%. The average recovery of norepinephrine was 94% with a range from 91 to 96%. In the second study, a standard solution of norepinephrine was added to several tissue extracts. The extracts were then carried through the analysis in the usual manner. Duplicate tissues were analyzed without the addition of norepinephrine. The difference in norepinephrine concentration between the duplicate samples was determined, and this value was compared with the known amount of norepinephrine added to the sample. In these experiments the average recovery of norepinephrine was 94% with a range from 90 to 98%.

All reagents used in this study were prepared with triple distilled water. All glassware was soaked in a solution of Alconox detergent, followed by a thorough washing with a test-tube brush. It was then rinsed several times in

tap water, followed by a rinsing in triple distilled water, and allowed to air-dry, inverted. A thorough rinsing is necessary in order that all traces of fluorescence introduced by the Alconox detergent may be removed. Originally, glassware was cleaned in sulfuric acid-potassium dichromate solution and cuvettes were boiled in a 1:1 mixture of concentrated nitric acid and water. However it was found that, with sufficient rinsing, the cleaning procedure described above with Alconox detergent was equally effective and far less cumbersome.

The instrument used in this study to measure fluorescence was the Coleman Model 12 C Electronic Photofluorometer. This same instrument has been used by other investigators in this field, e.g., von Euler (87) and Schaepdryver (238). The source of exciting light is a mercury lamp. A primary filter is placed between the lamp and the sample. In this study, three different primary filters were used. The first primary filter consisted of a Corning #5113 filter in combination with a Corning #3389 filter. It transmitted light of a wavelength from 415 mu. to 470 mu. The second primary filter was a Corning #5970 filter. It transmitted light of a wavelength from 320 mu. to 420 mu. The third primary was a Corning #5874 filter. It transmitted light of a wavelength from 310 mu. to 400 mu. A secondary filter is placed between the sample and the high vacuum phototube. The purpose of this filter is to pass the fluorescent light while rejecting the exciting light. In this study the secondary filter was a cut-off filter which rejected all light of wavelength below 490 mu. The fluorescent light from the sample produces

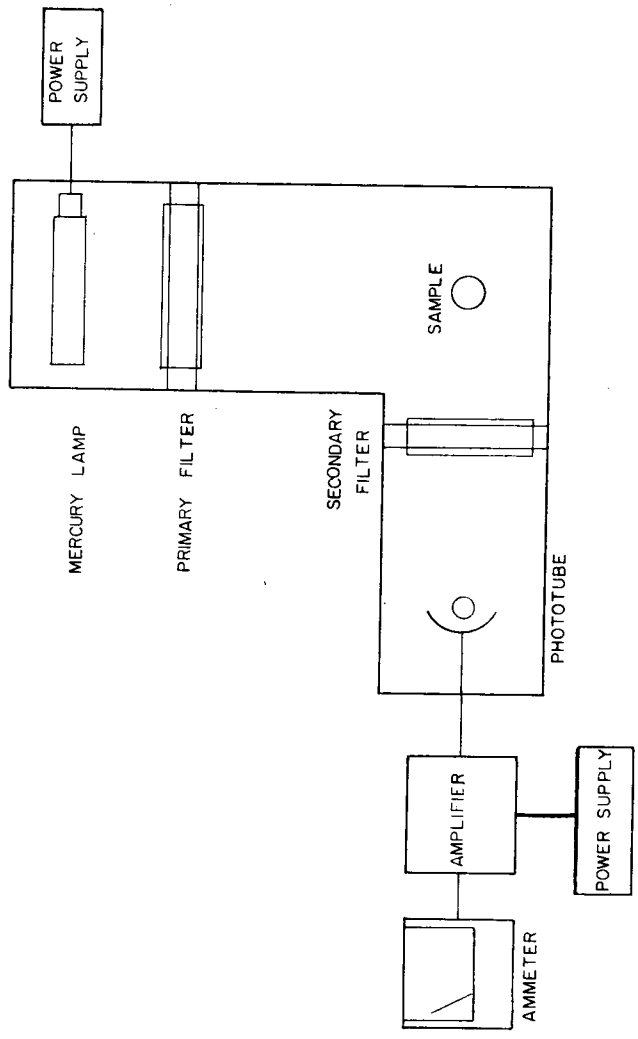
a response in the high vacuum phototube whose output is fed through an amplifier and then into a linear microammeter having a 100 division scale.

As a fluorescence standard, a solution of quinine sulfate was recommended by Lund and by von Euler. However, this substance was not used in this study since it was discovered that the wavelengths of the exciting and emitting light are such that the filter system described above could not be used. Erne recommended the use of a fluorescein solution (69). This was tried in this study but was later discarded when it was found that the fluorescein solution tended to fade with aging and could not be stored for any period of time. De Schaepdryver recommended the use of a plastic tube, fixed in a photofluorometer cuvette, as a fluorescence standard. This technique was tried and found to give reliable results. In this study, therefore, the fluorescence standard used was a plastic tube which was fixed in a cuvette.

The sensitivity of the fluorometer was set with the fluorescence standard prior to each reading. Standard solutions of epinephrine and norepinephrine were not analyzed with each tissue analysis, but were analyzed at various periods of time throughout this study.

Figure 3

SCHEMATIC OF COLEMAN MODEL 12C ELECTRONIC PHOTOFLUOROMETER



Calculation of the concentration of epinephrine and norepinephrine in each tissue sample was accomplished in the following manner. When standard solutions containing 0.20 u gm. epinephrine or 0.20 u gm. norepinephrine were analyzed and the fluorescence read at the three exciting wavelengths, the following data were obtained:

	436 mu Hg line	405 mu Hg line	366 mu Hg line
Epinephrine	48.0	49.0	26.0
Norepinephrine	14.0	48.5	26.5

The values reported represent net fluorescence, i.e., the reading of the sample minus the reading of the blank. Using these values, the amounts of epinephrine and norepinephrine which would give a full scale deflection (100 units) at each wavelength was calculated. These amounts were as follows:

	436 mu Hg line	405 mu Hg line	366 mu Hg line
Epinephrine	0.43 u gm.	0.41 u gm.	0.77 u gm.
Norepinephrine	1.43 u gm.	0.41 u gm.	0.75 u gm.

The tissue samples were likewise read at the three exciting wavelengths. To determine the total catecholamine concentration in the tissue sample, the net fluorescence of the sample at the 405 mu and the 366 mu wavelengths were used.

Using the 405 reading, the calculation is as follows:

$$\frac{(\text{Reading})(0.41)(10)}{(100)(\text{wt. of sample})} = \text{concentration total catecholamines}$$

Using the 366 reading, the calculation is as follows:

$$\frac{(\text{Reading})(0.76)(10)}{(100)(\text{wt. of sample})} = \text{concentration total catecholamines}$$

The factor of 10 is present in the numerator because only one-tenth of the eluate was analyzed. The factor 100 is present in the denominator because the amount of catecholamine which would give 100 units of deflection is used in the calculation. The reading used in these calculations is the average of the net fluorescence of the two aliquots of eluate which were analyzed. The values obtained from using the 405 reading and the 366 reading agree closely and the average of the two is taken as the concentration of the total catecholamines in the tissue sample. The duplicate tissue sample from the same area of the heart is analyzed in the same manner. The values for concentration of total catecholamines in the two tissue samples are then averaged and the resulting figure is reported for the concentration of total catecholamines in that particular area of the heart.

To differentiate between the amount of epinepinephrine and the amount of norepinephrine in the tissue sample, the following calculations were carried out. First it is assumed that all of the catecholamine in the tissue sample is norepinephrine. The calculation, using the 437 mu reading, is as follows:

$$\frac{(\text{Reading})(1.43)(10)}{(100)(\text{wt. of sample})} = \text{Concentration norepinephrine}$$

If the value obtained agrees with the value previously calculated for total catecholamine, it is reported as the concentration of norepinephrine in the tissue sample and the value for epinephrine is reported as zero. If, however, the value obtained is higher than the value previously calculated for total catecholamine, the following calculation is performed.

The calculation utilizing the 405 and 366 readings has given a value for $E + N$. The calculation utilizing the 436 reading has given a value for $\left[N + \frac{1.43}{0.42} E\right]$. The difference between these two calculations, therefore, is represented as $\left[N + \frac{1.43}{0.42} E\right] - \left[E+N\right]$ or 2.4E. In other words, the difference between the calculation with the 436 reading and the previous calculation for total catecholamine gives a value which is equal to 2.4 times the actual amount of epinephrine present in the sample. Therefore, the difference between these two calculations is now divided by 2.4 and the value obtained is reported as the concentration of epinephrine in the tissue sample. This value is subtracted from the value previously obtained for the concentration of total catecholamine to give the value for concentration of norepinephrine.

Price and Price have recommended the use of simultaneous equation in calculation the amount of epinephrine and of norepinephrine in a sample (218). However in this writer's experience, satisfactory results could not be obtained with this method of calculation. It seems that perhaps the experimental error inherent in the fluorimetric analysis of catecholamines is too great to

warrant such a precise mathematical analysis of the data.

The maximum fluorescence emitted by the lutine solution formed with epinephrine is at approximately 540 mu, while the maximum fluorescence emitted by the lutine solution formed with norepinephrine is at approximately 550 mu (131). The exact wavelengths of maximum fluorescence depend upon the wavelengths of exciting light. The most accurate way of analyzing epinephrine and norepinephrine is therefore with a spectrophotofluorometer. Then the spectral emission curves of the samples can be analyzed and compared with the spectral emission curves produced by standard solutions of epinephrine and norepinephrine. Since such an instrument was not available in this study, the fluorometer described above was used and the calculations were made in the manner described previously.

CHAPTER III

EXPERIMENTAL RESULTS

The animals used in this study were sacrificed at various periods of time after the initial operation. In all, a total of 64 animals were analyzed. Of these, eight were control animals and four were sham operated animals. On four of the animals a bilateral sympathectomy was performed. The remaining 52 animals underwent a unilateral cervico-thoracic sympathectomy. These animals were sacrificed between 2 days and 58 days following the initial operation.

Four-chamber pressure recordings were obtained for 27 of the unilaterally sympathectomized animals. These recordings were analyzed and the functional data were compared with the chemical data obtained from the catecholamine analysis. At one instance during this study, the samples from twelve hearts were lost due to a power failure in which the freezer went off and the frozen heart samples thawed. Functional recordings had previously been obtained from four of these twelve hearts. For these particular experiments, therefore, no comparison could be made between functional and chemical data.

A total of twelve tissue samples were analyzed from the majority of the hearts in this study. Two samples each were taken from 1) the right atrium, 2) the left atrium, 3) the right ventricular free wall, 4) the left ventricular free wall near the apex, 5) the left ventricular free wall near the base, and

6) the interventricular septum. On some hearts, only ten samples were analyzed, the left ventricular apex sample being omitted.

The following is a table of the chemical data obtained from each of the 68 hearts which were analyzed. The first column gives the area of the heart from which the sample was taken. In the second column is reported the content of catecholamines, i.e., epinephrine plus norepinephrine, reported as u gm per gram of tissue. The third column gives the content of epinephrine for each area of the heart; and the fourth column gives the content of norepinephrine, determined as the difference between columns two and three. The values reported in columns two and three were calculated in the manner described previously in the chapter on methods.

Table 1

Sample	E+N (u gm)/gm	E (u gm)/gm	N (u gm)/gm
Dog C-1, Control, 25 Kg.			
RA	1.29	0.00	1.29
LA	0.72	0.17	0.55
RV	0.42	0.08	0.34
(base) LV	0.34	0.04	0.30
S	0.29	0.03	0.26
Dog C-2, Control, 20 Kg.			
RA	1.53	0.00	1.53
LA	0.75	0.27	0.48
RV	0.60	0.11	0.49
(base) LV	0.56	0.14	0.42
S	0.50	0.07	0.43
Dog C-3, Control, 13 Kg.			
RA	1.44	0.08	1.36
LA	1.82	0.07	1.75
RV	0.77	0.17	0.60
(base) LV	0.87	0.18	0.69
S	0.70	0.18	0.52

Dog C-4, Control, 13 Kg.			
RA	1.62	0.00	1.62
LA	1.40	0.00	1.40
RV	0.81	0.06	0.75
(base) LV	0.43	0.03	0.40
S	0.49	0.13	0.36
Dog C-5, Control, 10 Kg.			
RA	1.24	0.04	1.20
LA	1.22	0.00	1.22
RV	0.88	0.00	0.88
(apex) LV	0.79	0.00	0.79
(base) LV	0.84	0.00	0.84
S	0.90	0.00	0.90
Dog C-6, Control, 10 Kg.			
RA	1.28	0.07	1.21
LA	1.44	0.29	1.15
RV	0.82	0.00	0.82
(apex) LV	0.60	0.00	0.60
(base) LV	0.72	0.00	0.72
S	0.71	0.00	0.71

Dog C-8, Control, 13 Kg.			
RA	0.90	0.01	0.89
LA	0.76	0.00	0.76
RV	0.74	0.06	0.68
(apex) LV	0.55	0.00	0.55
(base) LV	0.49	0.01	0.48
S	0.72	0.01	0.71
Dog C-10, Control, 8 Kg.			
RA	2.15	0.14	2.01
LA	0.93	0.00	0.93
RV	1.21	0.11	1.10
(apex) LV	1.10	0.01	1.09
(base) LV	0.74	0.01	0.73
S	1.00	0.13	0.87
Dog Sham-1, Right Side, 4 days, 14 Kg.			
RA	1.30	0.00	1.30
LA	1.58	0.17	1.41
RV	0.65	0.04	0.61
(apex) LV	0.53	0.08	0.45
(base) LV	0.49	0.08	0.41
S	0.48	0.04	0.44

Dog Sham-2, Right Side, 4 days, 12 Kg.

RA	2.10	0.17	1.93
LA	1.03	0.08	0.95
RV	0.78	0.03	0.75
(apex) LV	0.48	0.08	0.40
(base) LV	0.43	0.11	0.32
S	0.70	0.06	0.64

Dog Sham-3, Left Side, 5 days, 19 Kg.

RA	0.70	0.00	0.70
LA	0.79	0.00	0.79
RV	0.59	0.03	0.56
(apex) LV	0.46	0.04	0.42
(base) LV	0.47	0.06	0.41
S	0.48	0.03	0.45

Dog Sham-4, Left Side, 5 days, 12.5 Kg.

RA	1.50	0.00	1.50
LA	1,74	0.17	1.57
RV	0.90	0.04	0.86
(apex) LV	0.45	0.03	0.42
(base) LV	0.71	0.03	0.68
S	0.52	0.06	0.46

Dog WD-20, Left Stellatectomy, 4 days, 11.5 Kg.

RA	1.04	0.00	1.04
LA	0.86	0.00	0.86
RV	0.38	0.00	0.38
(base) LV	0.18	0.00	0.18
S	0.28	0.00	0.28

Dog WD-23, Left Stellatectomy, 2 days, 9.5 Kg.

RA	0.89	0.06	0.83
LA	1.32	0.17	1.15
RV	0.36	0.01	0.35
(apex) LV	0.26	0.00	0.26
(base) LV	0.26	0.01	0.25
S	0.21	0.00	0.21

Dog WD-25, Left Stellatectomy, 4 days, 7 Kg.

RA	1.30	0.00	1.30
LA	0.97	0.25	0.72
RV	0.29	0.08	0.21
(apex) LV	0.27	0.00	0.27
(base) LV	0.34	0.03	0.31
S	0.35	0.00	0.35

Dog WD-26, Left Stellatectomy, 3 days, 14 Kg.

RA	0.82	0.21	0.61
LA	0.54	0.17	0.37
RV	0.27	0.06	0.21
(apex) LV	0.38	0.06	0.32
(base) LV	0.31	0.00	0.31
S	0.44	0.00	0.44

Dog WD-28, Left Stellatectomy, 3 days, 10 Kg.

RA	1.02	0.10	0.92
LA	0.97	0.00	0.97
RV	0.47	0.04	0.43
(apex) LV	0.46	0.00	0.46
(base) LV	0.34	0.00	0.34
S	0.49	0.00	0.49

Dog APR-29, Left Stellatectomy, 3 days, 12 Kg.

RA	0.93	0.28	0.65
LA	0.25	0.13	0.12
RV	0.49	0.13	0.36
(apex) LV	0.60	0.10	0.50
(base) LV	0.55	0.07	0.48
S	0.38	0.00	0.38

Dog APR-31, Left Stellatectomy, 3 days, 12 Kg.

RA	0.54	0.00	0.54
LA	1.00	0.00	1.00
RV	0.75	0.00	0.75
(apex) LV	0.56	0.11	0.45
(base) LV	0.30	0.13	0.17
S	0.51	0.01	0.50

Dog APR-34, Left Stellatectomy, 4 days, 11 Kg.

RA	0.90	0.00	0.90
LA	1.27	0.00	1.27
RV	0.29	0.01	0.28
(apex) LV	0.40	0.00	0.40
(base) LV	0.19	0.04	0.15
S	0.31	0.00	0.31

Dog APR-35, Left Stellatectomy, 4 days, 13 Kg.

RA	1.10	0.07	1.03
LA	1.15	0.00	1.15
RV	0.61	0.00	0.61
(apex) LV	0.38	0.00	0.38
(base) LV	0.35	0.00	0.35
S	0.49	0.04	0.45

Dog WD-21, Right Stellatectomy, 4 days, 12 Kg.

RA	0.66	0.00	0.66
LA	0.47	0.06	0.41
RV	0.38	0.00	0.38
(apex) LV	0.32	0.00	0.32
(base) LV	0.19	0.00	0.19
S	0.43	0.06	0.37

Dog WD-22, Right Stellatectomy, 3 days, 12 Kg.

RA	0.70	0.00	0.70
LA	0.88	0.08	0.80
RV	0.73	0.00	0.73
(apex) LV	0.58	0.00	0.58
(base) LV	0.45	0.01	0.44
S	0.46	0.00	0.46

Dog WD-24, Right Stellatectomy, 4 days, 9.5 Kg.

RA	0.50	0.00	0.50
LA	1.06	0.25	0.81
RV	0.36	0.00	0.36
(apex) LV	0.39	0.00	0.39
(base) LV	0.35	0.00	0.35
S	0.24	0.00	0.24

Dog WD-27, Right Stellatectomy, 3 days, 10.5 Kg.

RA	1.24	0.00	1.24
LA	1.77	0.10	1.67
RV	0.39	0.00	0.39
(apex) LV	0.43	0.14	0.29
(base) LV	0.39	0.04	0.35
S	0.53	0.00	0.53

Dog WD-29, Right Stellatectomy, 3 days, 12.5 Kg.

RA	0.93	0.21	0.72
LA	1.31	0.28	1.03
RV	0.87	0.00	0.87
(apex) LV	0.70	0.06	0.64
(base) LV	0.52	0.10	0.42
S	0.41	0.04	0.37

Dog APR-30, Right Stellatectomy, 5 days, 15 Kg.

RA	0.16	0.08	0.08
LA	0.17	0.08	0.09
RV	0.25	0.00	0.25
(apex) LV	0.27	0.00	0.27
(base) LV	0.45	0.00	0.45
S	0.38	0.00	0.38

Dog APR-32, Right Stellatectomy, 3 days, 14 Kg.

RA	0.23	0.17	0.06
LA	0.49	0.08	0.41
RV	0.48	0.00	0.48
(apex) LV	0.33	0.00	0.33
(base) LV	0.32	0.00	0.32
S	0.39	0.03	0.36

Dog APR-36, Right Stellatectomy, 3 days, 12 Kg.

RA	0.42	0.04	0.38
LA	0.67	0.14	0.53
RV	0.34	0.21	0.13
(apex) LV	0.30	0.11	0.19
(base) LV	0.37	0.04	0.33
S	0.38	0.07	0.31

Dog APR-37, Right Stellatectomy, 3 days, 10 Kg.

RA	0.46	0.00	0.46
LA	0.73	0.06	0.67
RV	0.68	0.03	0.65
(apex) LV	0.48	0.06	0.42
(base) LV	0.55	0.07	0.48
S	0.44	0.02	0.42

Dog WD-13, Left Stellatectomy, 7 days, 14 Kg.

RA	0.87	0.08	0.79
LA	0.96	0.11	0.85
RV	0.49	0.00	0.49
(base) LV	0.23	0.00	0.23
S	0.36	0.00	0.36

Dog WD-15, Left Stellatectomy, 7 days, 8.5 Kg.

RA	1.14	0.03	1.11
LA	1.08	0.25	0.83
RV	0.52	0.00	0.52
(base) LV	0.30	0.00	0.30
S	0.38	0.00	0.38

Dog WD-18, Left Stellatectomy, 7 days, 14 Kg.

RA	0.75	0.06	0.69
LA	0.40	0.00	0.40
RV	0.39	0.00	0.39
(apex) LV	0.31	0.00	0.31
(base) LV	0.31	0.00	0.31
S	0.33	0.00	0.33

Dog WD-19, Left Stellatectomy, 7 days, 7.5 Kg.

RA	-	-	-
LA	1.12	0.07	1.05
RV	0.79	0.00	0.79
(base) LV	0.68	0.00	0.68
S	0.54	0.00	0.54

Dog APR-19, Left Stellatectomy, 7 days, 9 Kg.

RA	0.93	0.18	0.75
LA	0.58	0.08	0.50
RV	0.41	0.00	0.41
(base) LV	0.43	0.00	0.43
S	0.41	0.00	0.41

Dog APR-21, Left Stellatectomy, 7 days, 15.5 Kg.

RA	0.35	0.01	0.34
LA	0.62	0.03	0.59
RV	0.18	0.00	0.18
(apex) LV	0.16	0.00	0.16
(base) LV	0.13	0.00	0.13
S	0.15	0.03	0.12

Dog APR-24, Left Stellatectomy, 7 days, 14 Kg.

RA	0.83	0.14	0.69
LA	0.48	0.03	0.45
RV	0.27	0.00	0.27
(apex) LV	0.35	0.00	0.35
(base) LV	0.28	0.00	0.28
S	0.31	0.00	0.31

Dog APR-33, Left Stellatectomy, 6 days, 8 Kg.

RA	0.89	0.27	0.62
LA	0.73	0.18	0.55
RV	0.37	0.00	0.37
(apex) LV	0.33	0.00	0.33
(base) LV	0.39	0.00	0.39
S	0.46	0.00	0.46

Dog WD-16, Right Stellatectomy, 7 days, 13 Kg.

RA	0.83	0.08	0.75
LA	1.38	0.25	1.13
RV	0.70	0.03	0.67
(apex) LV	0.73	0.00	0.73
(base) LV	0.78	0.00	0.78
S	0.70	0.00	0.70

Dog WD-17, Right Stellatectomy, 7 days, 10 Kg.

RA	0.77	0.17	0.60
LA	1.76	0.13	1.63
RV	0.90	0.04	0.86
(apex) LV	0.68	0.01	0.67
(base) LV	0.74	0.01	0.73
S	0.66	0.00	0.66

Dog APR-20, Right Stellatectomy, 7 days, 8.5 Kg.

RA	0.32	0.07	0.25
LA	0.42	0.00	0.42
RV	0.09	0.00	0.09
(apex) LV	0.20	0.00	0.20
(base) LV	0.19	0.00	0.19
S	0.18	0.00	0.18

Dog APR-22, Right Stellatectomy, 7 days, 5.5 Kg.

RA	0.74	0.04	0.70
LA	0.62	0.10	0.52
RV	0.42	0.07	0.35
(apex) LV	0.43	0.06	0.37
(base) LV	0.72	0.07	0.65
S	0.63	0.03	0.60

Dog APR-23, Right Stellatectomy, 7 days, 9.5 Kg.			
RA	0.99	0.11	0.88
LA	0.93	0.08	0.85
RV	0.19	0.01	0.18
(apex) LV	0.40	0.00	0.40
(base) LV	0.58	0.00	0.58
S	0.49	0.01	0.48
Dog WD-12, Left Stellatectomy, 14 days, 11 Kg.			
RA	0.76	0.11	0.65
LA	0.94	0.11	0.83
RV	0.29	0.08	0.21
(base) LV	0.09	0.08	0.01
S	0.23	0.06	0.17
Dog APR-17, Left Stellatectomy, 14 days, 11 Kg.			
RA	1.66	0.00	1.66
LA	1.05	0.29	0.76
RV	1.08	0.00	1.08
(base) LV	0.91	0.00	0.91
S	0.83	0.00	0.83

Dog APR-18, Left Stellatectomy, 16 days, 10.5 Kg.

RA	1.38	0.01	1.37
LA	1.09	0.27	0.82
RV	0.57	0.08	0.49
(base) LV	0.35	0.08	0.27
S	0.31	0.08	0.23

Dog WD-4, Left Stellatectomy, 30 days, 9 Kg.

RA	1.65	0.13	1.52
LA	1.40	0.31	1.09
RV	0.48	0.11	0.37
(base) LV	0.50	0.07	0.43
S	0.57	0.08	0.49

Dog APR-16, Left Stellatectomy, 29 days, 11 Kg.

RA	2.00	0.00	2.00
LA	0.56	0.13	0.43
RV	0.37	0.00	0.37
(base) LV	0.11	0.00	0.11
S	0.28	0.00	0.28

Dog APR-13, Right Stellatectomy, 14 days, 8.5 Kg.

RA	0.35	0.22	0.13
LA	0.48	0.21	0.27
RV	0.37	0.24	0.13
(base) LV	0.41	0.14	0.27
S	0.45	0.21	0.24

Dog APR-14, Right Stellatectomy, 14 days, 7 Kg.

RA	1.80	0.18	1.62
LA	0.98	0.55	0.43
RV	0.46	0.14	0.32
(base) LV	0.36	0.10	0.26
S	0.28	0.14	0.14

Dog WD-3, Right Stellatectomy, 33 days, 10 Kg.

RA	0.66	0.00	0.66
LA	1.25	0.00	1.25
RV	0.46	0.00	0.46
(base) LV	0.36	0.00	0.36
S	0.40	0.00	0.40

Dog APR-15, Right Stellatectomy, 29 days, 12 Kg.

RA	0.81	0.00	0.81
LA	1.14	0.11	1.03
RV	0.41	0.00	0.41
(base) LV	0.80	0.00	0.80
S	0.38	0.00	0.38

Dog WD-1, Left Stellatectomy, 41 days, 9 Kg.

RA	1.37	0.29	1.08
LA	1.02	0.14	0.88
RV	0.70	0.00	0.70
(base) LV	0.49	0.00	0.49
S	0.50	0.03	0.47

Dog WD-7, Left Stellatectomy, 51 days, 16 Kg.

RA	1.24	0.29	0.95
LA	2.54	0.13	2.41
RV	0.73	0.00	0.73
(base) LV	0.49	0.07	0.42
S	0.56	0.07	0.49

Dog APR-1, Left Stellatectomy, 44 days, 11 Kg.

RA	1.25	0.24	1.01
LA	1.98	0.10	1.88
RV	0.71	0.03	0.68
(base) LV	0.49	0.01	0.48
S	0.88	0.01	0.87

Dog APR-4, Left Stellatectomy, 45 days, 19 Kg.

RA	1.17	0.04	1.13
LA	1.70	0.32	1.38
RV	0.58	0.04	0.54
(base) LV	0.71	0.00	0.71
S	0.58	0.03	0.55

Dog WD-2, Right Stellatectomy, 42 days, 15 Kg.

RA	1.37	0.00	1.37
LA	0.88	0.00	0.88
RV	-	-	-
(base) LV	0.53	0.01	0.52
S	0.46	0.00	0.46

Dog WD-6, Right Stellatectomy, 42 days, 15 Kg.

RA	1.69	0.00	1.69
LA	1.36	0.00	1.36
RV	1.63	0.00	1.63
(base) LV	0.75	0.00	0.75
S	0.80	0.00	0.80

Dog APR-2, Right Stellatectomy, 43 days, 10 Kg.

RA	2.01	0.00	2.01
LA	2.19	0.06	2.13
RV	1.03	0.13	0.90
(base) LV	0.67	0.11	0.56
S	0.59	0.08	0.51

Dog APR-3, Right Stellatectomy, 43 days, 11 Kg.

RA	0.83	0.07	0.76
LA	0.91	0.17	0.74
RV	0.24	0.00	0.24
(base) LV	0.98	0.04	0.94
S	0.48	0.13	0.35

Dog APR-25, Bilateral, 8 days post right and 2 days post left, 9.5 Kg.

RA	0.28	0.08	0.20
LA	0.39	0.01	0.38
RV	0.35	0.00	0.35
(apex) LV	0.25	0.00	0.25
(base) LV	0.24	0.00	0.24
S	0.21	0.00	0.21

Dog APR-26, Bilateral, 16 days post right and 2 days post left, 10 Kg.

RA	0.37	0.08	0.29
LA	1.02	0.25	0.77
RV	0.45	0.00	0.45
(apex) LV	0.22	0.00	0.22
(base) LV	0.31	0.00	0.31
S	0.19	0.00	0.19

Dog APR-27, Bilateral, 9 days post right and 2 days post left, 13 Kg.

RA	1.41	0.13	1.28
LA	1.95	0.25	1.70
RV	0.67	0.08	0.59
(apex) LV	0.42	0.00	0.42
(base) LV	0.30	0.00	0.30
S	0.59	0.03	0.56

Dog APR-28, Bilateral, 15 days post right and 2 days post left, 12 Kg.

RA	0.74	0.11	0.63
LA	0.69	0.14	0.55
RV	0.32	0.00	0.32
(apex) LV	0.60	0.00	0.60
(base) LV	0.57	0.00	0.57
S	0.66	0.00	0.66

The data from the control hearts demonstrate clearly that there is a distinct difference between the catecholamine content of the atria and that of the ventricles. Without a single exception, the catecholamine level was markedly higher in the atrium than in the ipsilateral ventricle. In most of the hearts, the level was higher in the right atrium than in the left, and it was also generally higher in the right ventricle than in the left. The differences between right and left atria and between right and left ventricles did not prove to be statistically significant. The p value for both comparisons was approximately 0.10. However, the difference between the combined atria and combined ventricles was statistically significant. The p value was less than 0.001.

In the data reported for the sham-operated animals, the atrial catecholamine level was, in every instance, higher than that of the ipsilateral ventricle. An analysis of the data from the unilaterally sympathectomized animals shows that the level in the atrium was higher than that in the ipsilateral ventricle in all but 9 out of 104 comparisons.

It is evident from a perusal of the preceding table that great variability from one animal to another exists in the range of values observed. The following table represents an attempt to simplify the data by reporting the average values obtained for each group of animals.

Table 2

Averaged Data

	RA	LA	RV	LVA	LVB	S
Controls (8)	1.46	1.13	0.78	0.64	0.62	0.67
Sham (4)	1.40	1.29	0.73	0.48	0.53	0.55
2-5 days (9) Lt.	0.90	0.93	0.43	0.41	0.34	0.38
2-5 days (9) Rt.	0.59	0.84	0.50	0.42	0.40	0.41
7 days (8) Lt.	0.86	0.70	0.42	0.28	0.34	0.36
7 days (5) Rt.	0.70	1.02	0.46	0.49	0.60	0.52
14-30 days (5) Lt.	1.53	1.01	0.56		0.39	0.45
14-33 days (4) Rt.	0.91	0.96	0.43		0.48	0.38
41-51 days (4) Lt.	1.26	1.81	0.68		0.55	0.63
42-43 days (4) Rt.	1.46	1.30	0.97		0.73	0.58
Bilaterals (4)	0.70	1.01	0.45	0.37	0.36	0.41

Values reported are µ gm of catecholamines (epinephrine plus norepinephrine) per gram of tissue in 1) the right atrium, 2) the left atrium, 3) the right ventricle, 4) the left ventricle near the apex, 5) the left ventricle near the base, and 6) the interventricular septum. Numbers in parentheses indicate the number of animals in each group. The symbols Lt. and Rt. indicate whether a left stellatectomy or a right stellatectomy was performed. At the extreme left of the chart is indicated the length of time which was allowed to elapse after the initial operation before the animals were sacrificed.

The catecholamine values in the sham-operated animals were not significantly different from those in the control animals. This indicates that the operation itself had no effect on the catecholamine content of the heart. The most marked reduction in catecholamine levels occurred in the animals studied one week or less after the initial operation. In the animals studied at approximately six weeks after the initial operation, the levels were not much different from those in the control animals.

In order to compare the effect of right sympathectomy with that of left sympathectomy, Tables 3 and 4 were prepared. The catecholamine levels in the sympathectomized animals were calculated as per cent of levels in the control animals.

Table 3

Catecholamine Levels Following Left Sympathectomy

	2-5 days	7 days	14-30 days	41-51 days
RA	62%	52%	105%	86%
LA	82%	62%	90%	160%
RV	55%	54%	72%	87%
LVA	64%	44%		
LVB	55%	55%	63%	90%
S	57%	54%	68%	94%

Table 4

Catecholamine Levels Following Right Sympathectomy

	2-5 days	7 days	14-33 days	42-43 days
RA	40%	48%	62%	100%
LA	74%	90%	85%	115%
RV	64%	60%	55%	124%
LVA	66%	77%		
LVB	65%	97%	80%	118%
S	61%	78%	57%	87%

It can be seen that left sympathectomy decreases the catecholamine level throughout the heart, the left atrium being the area least affected. On the other hand, right sympathectomy decreases the level mainly in the right atrium and the right ventricle, the left atrium and left ventricle being less seriously affected.

Tables 5 and 6 were prepared in order to compare the chemical data with the functional data obtained from analysis of the four chamber pressure recordings. Column one gives the number of the animal. Column two indicates the length of time which had elapsed since the initial operation. In column three, the contractile response of the right atrium is reported as the percentage change in the A-wave during stimulation as compared with the pre-stimulation value. Immediately under the contractile response is reported the content of norepinephrine in the right atrium calculated as u gm per gram of tissue. Column four shows the contractile response of the right ventricle, reported as the percentage change during stimulation. Below the contractile response, the norepinephrine content of the right ventricle is given. In column five are shown the same data for the left atrium as are shown in column three for the right atrium. Column six gives the data for the left ventricle. In column seven is reported the absolute change in heart rate from before stimulation to during stimulation. For animals WD 8, WD 9, WD 10 and WD 11, no chemical data was available.

Table 5

Left Stellatectomy Followed by Right Stellate Stimulation

Animal	Days post op.	RA	RV	LA	LV	Heart Rate
WD 11	58	-35 -	+ 300 -	+ 25 -	+ 20 -	170-240
WD 7	51	+ 400 1.03	+ 90 0.73	+ 300 2.45	-15 0.44	140-270
WD 8	45	+ 85 -	+ 135 -	+ 125 -	+ 110 -	190-210
WD 1	41	+ 50 1.16	+ 60 0.70	+ 15 0.92	+ 15 0.49	130-130
WD 4	31	+ 125 1.56	+ 45 0.40	+ 130 1.18	+ 25 0.45	185-215
WD 12	14	+ 115 0.68	+ 200 0.23	+ 85 0.86	+ 95 0.03	180-250
WD 13	7	+100 0.81	+ 65 0.49	+ 270 0.88	+ 35 0.23	180-270
WD 15	7	0 1.12	+ 35 0.52	0 0.90	0 0.30	170-240
WD 18	7	0 0.71	+ 60 0.39	+ 20 0.40	+ 35 0.31	190-240
WD 19	7	+ 140 -	+ 70 0.79	+ 25 1.07	+ 75 0.68	195-240
WD 20	4	+100 1.04	0 0.38	0 0.86	0 0.18	210-250
WD 23	2	+ 50 0.85	+10 0.35	-80 1.20	0 0.25	85-205
WD 25	4	+ 35 1.30	+250 0.23	+ 50 0.79	+ 15 0.32	205-240
WD 26	3	+ 25 0.67	+70 0.23	0 0.42	+ 20 0.31	145-180
WD 28	3	+ 300 0.95	+15 0.44	+ 70 0.97	+ 15 0.34	110-190

Table 6

Right Stellatectomy Followed by Left Stellate Stimulation

Animal	Days post op.	RA	RV	LA	LV	Heart Rate
WD 9	49	+115 -	+180 -	+150 -	+30 -	200-220
WD 4	42	+15 1.37	+10 -	-25 0.88	-10 0.52	115-140
WD 6	42	+30 1.69	+30 1.63	+20 1.36	+5 0.75	170-205
WD 3	33	+100 0.66	+100 0.46	+250 1.25	+65 0.36	160-210
WD 10	15	-50 -	+100 -	-100 -	+30 -	190-220
WD 16	7	+75 0.77	+100 0.68	0 1.20	+20 0.78	150-210
WD 17	7	0 0.65	+25 0.87	0 1.67	+10 0.73	160-160
WD 21	4	+100 0.66	+10 0.38	0 0.43	0 0.19	165-170
WD 22	3	0 0.70	0 0.73	0 0.82	0 0.44	160-160
WD 24	4	0 0.50	+100 0.36	+65 0.88	+125 0.35	155-190
WD 27	3	0 1.24	+225 0.39	0 1.70	+185 0.36	230-240
WD 29	3	+400 0.78	+75 0.87	+15 1.11	+45 0.38	140-150

A correlation between the functional response of the animal and the cardiac norepinephrine content was not present in every experiment. Many of the animals whose hearts were relatively depleted of norepinephrine gave good functional responses to stimulation, while other animals whose hearts retained significant amounts of norepinephrine gave a poor response or none at all.

The polygraph records reproduced on the following two pages are four chamber pressure recordings obtained from the hearts of open-chested mongrel dogs in which either the right or the left stellate ganglion was stimulated. Figure 4 is a record from a control non-sympathectomized animal in which the left stellate ganglion was stimulated. The tracings show an immediate and profound increase in pressures recorded from all four chambers. This record is typical of the response of the control dog to left stellate stimulation. Figure 5 represents the response of a control non-sympathectomized animal to stimulation of the right stellate ganglion. Acceleration of the heart rate was more prominent and the left ventricular pressure elevation was less marked during a right stellate stimulation than with stimulation of the left stellate ganglion. In this figure the mean pressures were recorded alternately with the pulsatile pressures. Figures 6, 7, and 8 are from animals which have undergone a unilateral sympathectomy. Figure 6 shows the pressure recordings from a dog on which a right stellatectomy had been performed 49 days previously (Animal #WD-9). Mean pressures in the four chambers were recorded alternately with the full

pressure pulses. At the signal, the intact left stellate ganglion was stimulated. The overall response was quite similar to the response observed in control non-sympathectomized dogs. The recordings show a prompt increase in pressure in all four chambers. There was also a slight increase in heart rate.

Figure 7 is a four chamber pressure recording from a dog which had undergone a right stellatectomy 15 days previously (Animal #WD-10). At the signal the intact left stellate ganglion was stimulated as above. In this animal, however, the responses were quite different. The right atrium showed an augmentation of the A-wave with little change in the mean pressure. The right ventricle was strongly augmented. The left atrium showed a depression with a fall in mean pressure. In the left ventricle there was practically no augmentation. The response of this heart to stellate stimulation was definitely abnormal.

Figure 8 shows the four chamber pressure recordings from a dog on which a left stellatectomy had been performed 14 days previously (Animal #WD-12). The fast records were taken at 10 times the speed of the slow records. At the signal the intact right stellate ganglion was stimulated. The most noticeable characteristic of this record is the very slow onset of the response to stellate stimulation. There was eventually an augmentation in all four chambers but it began late and developed very gradually. This is definitely an abnormal response to stellate stimulation.

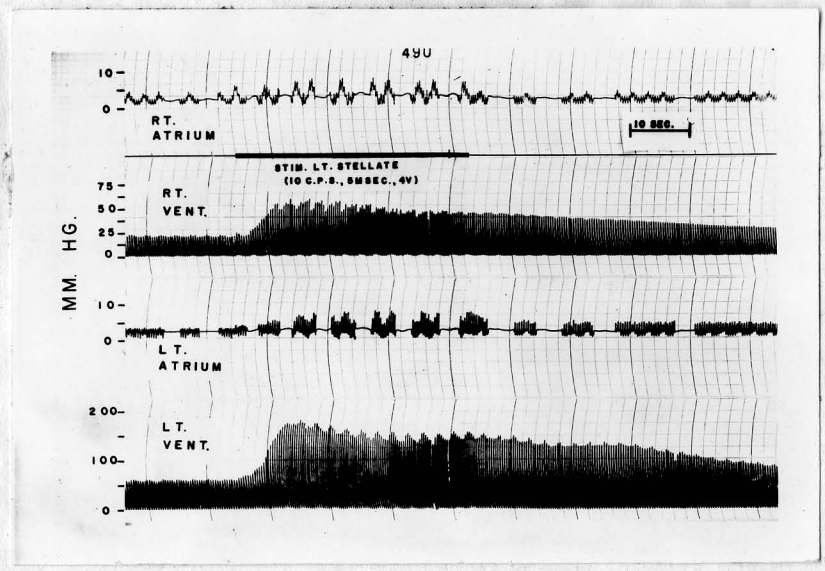


Figure 4

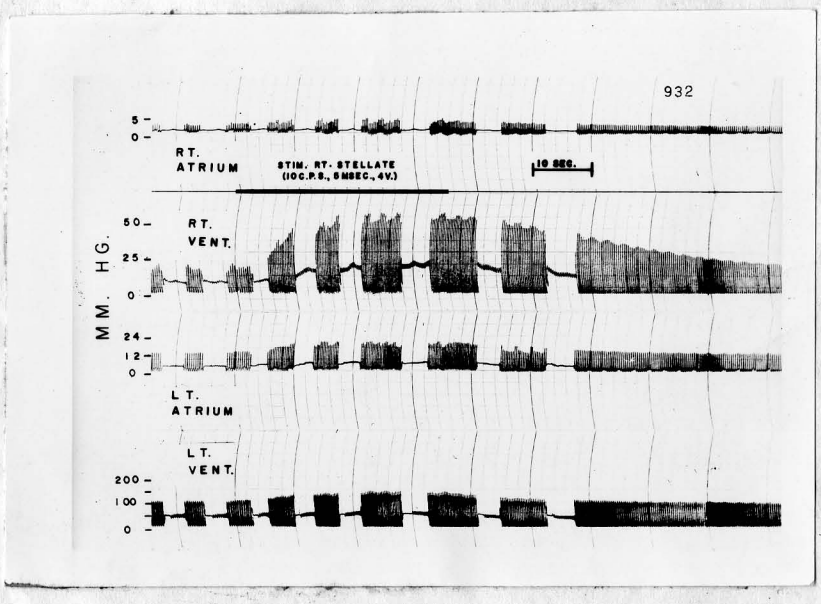


Figure 5

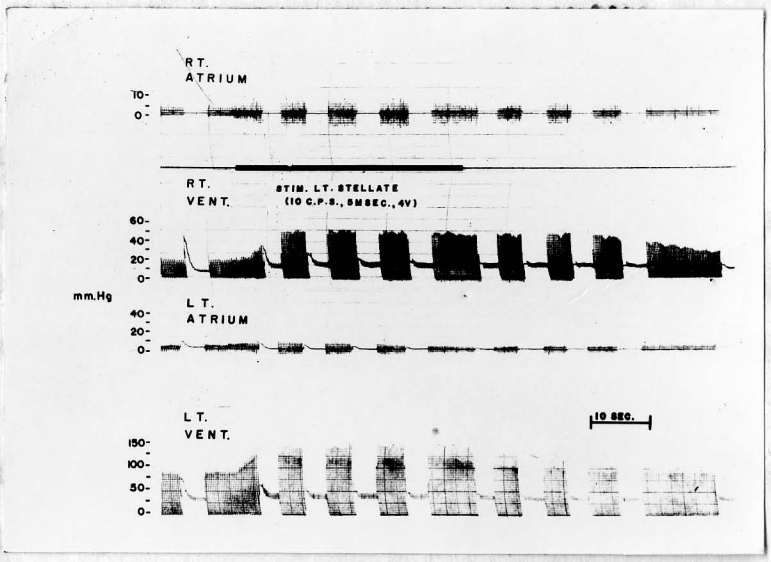


Figure 6

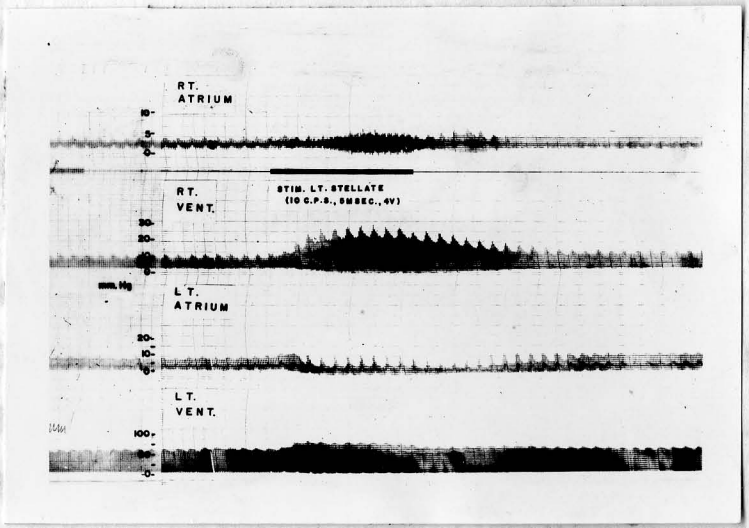


Figure 7

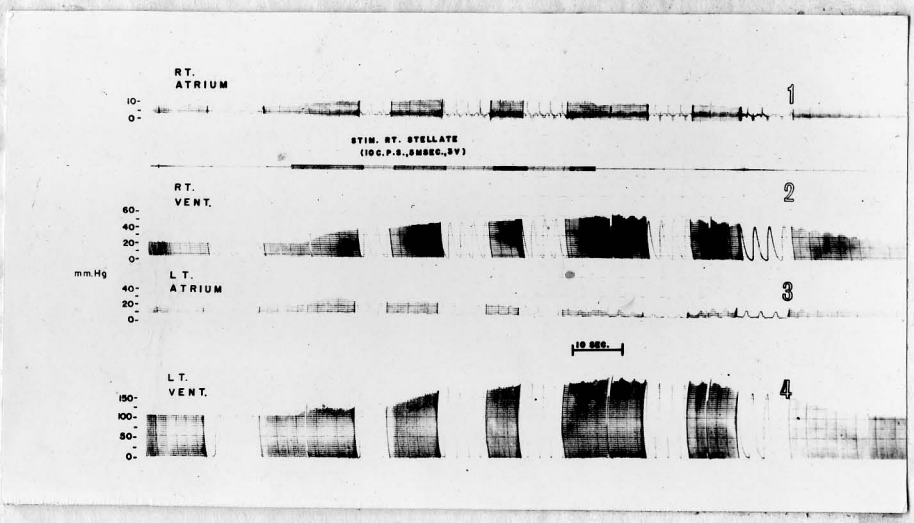


Figure 8

The records shown in figures 7 and 8 are by no means typical of animals who have undergone either a right or a left cardiac sympathectomy. The responses observed are extremely variable from animal to animal. An individual analysis of the functional records reveals the following information:

A. Stimulation of the right stellate ganglion in animals which had previously undergone a left stellatectomy.

WD 11 - The responses to stimulation were immediate but lasted only a few seconds in all chambers except the right ventricle. In the other three chambers the responses disappeared even though the stimulus was still being applied.

WD 7 - The responses to stimulation were immediate and returned to control levels approximately 50 seconds after cessation of the stimulation. The left ventricle responded with a depression in amplitude of contraction.

WD 8 - The responses to stimulation were immediate. However, they ceased abruptly when the stimulation was ended. There was no gradual return to control levels.

WD 1 - The responses to stimulation developed slowly and required 25 seconds to attain their maximum. The responses observed were small.

WD 4 - The responses to stimulation required 35 seconds to reach their maximal level. In the left ventricle there was an initial depression in systolic pressure.

WD 12 - The responses obtained were good but required 30 seconds to

reach their maximum.

WD 13 - The responses were good but developed gradually and required 30 seconds to attain maximum values.

WD 15 - Only the right ventricle responded and its response was delayed 15 seconds after the beginning of stimulation.

WD 18 - The responses were small and were delayed 25 seconds after the beginning of stimulation.

WD 19 - The responses were immediate and well developed.

WD 20 - Only the right ventricle responded.

WD 23 - No response occurred in the left ventricle. The responses in the other chambers were immediate. The left atrium responded with a depression in systolic pressure.

WD 25 - The responses developed gradually over 15 seconds.

WD 26 - Only the right ventricle responded well.

WD 28 - The responses were good and attained their maximum in 10 seconds.

B. Stimulation of the left stellate ganglion in animals which had previously undergone a right stellatectomy.

WD 9 - The responses were good and looked much like those obtained from a non-sympathectomized animal.

WD 2 - The responses were very small and required 20 seconds to reach their maximum. The response in the left atrium and ventricle was a depression in systolic pressure.

WD 6- The responses were immediate but small and lasted only a few seconds

even though the stimulus was still being applied.

WD 3 - The responses were good but required 20 seconds to reach their maximum. The return to control conditions required over 1 1/2 minutes.

WD 10 - The response in both atria was a depression in systolic pressure. Return to control conditions in the ventricles was slow.

WD 16 - The attainment of maximum response required nearly 1 minute. The return to control conditions took more than a minute.

WD 17 - The responses were very small but developed immediately.

WD 21 - Only the right atrium gave a good response.

WD 22 - There was no response at all.

WD 24 - The responses required 30 seconds to reach maximum. Return to control required more than a minute.

WD 27 - Neither atrium responded. The responses in the ventricles were good and developed immediately.

WD 29 - Good responses developed promptly in every chamber. The return to control conditions required 1 1/2 minutes.

CHAPTER IV

GENERAL DISCUSSION

The heart is supplied with sympathetic nerve fibers from both the right and the left sympathetic trunks by way of the corresponding stellate and caudal cervical ganglia. It has been suggested that the right cervicothoracic trunk furnishes the primary sympathetic innervation of the right heart while the left sympathetics supply mainly the left heart. This relationship was suggested in 1902 by von Schumacher in a report of a series of dissection studies (244).

The mass of functional data reported on stimulation of the right and the left sympathetic nerve supplies also seems to indicate a difference in the distribution to the heart from the right sympathetic trunk as compared with the distribution from the left sympathetic trunk. The effect on the heart rate is, on the average, more pronounced with a right sympathetic stimulation than with a left, while the effect on ventricular contraction is more prominent with a left sympathetic stimulation. This distinction is by no means absolute and there is considerable variation from animal to animal.

This variability in response between animals is evident also in studies on unilaterally sympathectomized animals and makes a precise analysis of the data somewhat difficult. There is no particular pattern which can be set down as the "typical" response of animals with a right or a left cardiac sympathectomy. However, in nearly every experiment, the response of the

animal can be designated as abnormal when analyzed in light of the average response usually obtained in the control, non-sympathectomized animal. In an open-chested, non-sympathectomized animal, stimulation of the left stellate ganglion produces an immediate and profound elevation of intraventricular pressures and of atrial pressures as well. There is also a slight increase in heart rate. The response is maintained during the course of the stimulation and gradually returns to control levels after the stimulus is removed.

If the same procedure is followed with an animal which has previously undergone a right cardiac sympathectomy, a different response is obtained. The most frequently seen deviation from the normal response pattern is an abnormally long delay in the attainment of the maximum response to stimulation. Instead of the prompt rise in pressure observed in the control animal, there is a slow and gradual increase in pressure which may or may not reach a level comparable to that observed in the non-sympathectomized animal. Such a delay can be demonstrated in the majority of experiments on unilaterally sympathectomized animals.

Another deviation frequently seen is an abnormally long period of time after cessation of the stimulation before the response returns to control levels. The response sometimes remains at its maximum value for approximately 30 seconds after the stimulus is removed before it begins to decline and then gradually returns to control levels.

In a few experiments, an animal with a left cardiac sympathectomy

showed essentially no response at all in the left atrium and ventricle and a relatively normal response in the right atrium and ventricle. And, vice versa, an animal having a right cardiac sympathectomy sometimes showed no response in the right atrium and ventricle. This would seem to indicate that, in these animals, removal of the left sympathetic supply denervated the left side of the heart while removal of the right sympathetic supply denervated the right side of the heart. These results would be in accord with the relationship suggested by von Schumacher.

However, in many of the experiments a unilateral sympathectomy altered the response to stellate stimulation in all four chambers of the heart. Many variations in response could be noted. The responses in all four chambers may be equally depressed or they may be much more depressed on the ipsilateral side. And in some cases the greatest depression of response was seen on the side of the heart opposite to the side of the denervation. These experiments indicate that the entire heart is supplied with sympathetic fibers from both the right and the left side. There seems however to be a great amount of variation from animal to animal.

A similar variability between animals was noted when the results of the chemical analyses were studied. A statistical analysis was applied to the data obtained from the chemical determinations. The standard deviation for each group was calculated and the values obtained are reported in Table 7. The values reported are u gm of catecholamines per gram of tissue. The numbers in parentheses indicate the standard deviation.

Table 7

	RA	LA	RV	LVA	LVB	S
Controls	1.46 (0.33)	1.13 (0.40)	0.78 (0.23)	0.64 (0.25)	0.62 (0.20)	0.67 (0.23)
Sham	1.40 (0.57)	1.29 (0.45)	0.73 (0.14)	0.43 (0.02)	0.53 (0.13)	0.55 (0.10)
2-5 Days Lt.	0.90 (0.21)	0.93 (0.33)	0.43 (0.16)	0.41 (0.12)	0.34 (0.11)	0.38 (0.11)
2-5 Days Rt.	0.59 (0.34)	0.84 (0.49)	0.50 (0.21)	0.42 (0.15)	0.40 (0.11)	0.41 (0.08)
7 Days Lt.	0.86 (0.24)	0.70 (0.53)	0.42 (0.18)	0.28 (0.08)	0.34 (0.16)	0.36 (0.11)
7 Days Rt.	0.70 (0.25)	1.02 (0.55)	0.46 (0.34)	0.49 (0.22)	0.60 (0.24)	0.52 (0.21)
2-4 Wks. Lt.	1.53 (0.46)	1.01 (0.30)	0.56 (0.31)		0.39 (0.30)	0.45 (0.20)
2-4 Wks. Rt.	0.91 (0.62)	0.96 (0.33)	0.43 (0.06)		0.48 (0.22)	0.38 (0.08)
6-7 Wks. Lt.	1.26 (0.08)	1.81 (0.63)	0.68 (0.06)		0.55 (0.10)	0.63 (0.16)
6-7 Wks. Rt.	1.46 (0.50)	1.30 (0.60)	0.97 (0.69)		0.73 (0.18)	0.58 (0.15)

The high standard deviation values indicate the large range in the data for any one group. In order to determine whether the difference between various groups are significant, the p-values were calculated. In each case, the catecholamine level of the control group was compared with the catecholamine level of the experimental group. Table 8 reports the p-values which were obtained.

Table 8

Control VS.	RA	LA	RV	LVA	LVB	S
1.) Sham	.900<p	.500<p<.600	.600<p<.700	.050<p<.100	.300<p<.400	.300<p<.400
2.) 2-5 days Lt.	p<.001*	.200<p<.300	.001<p<.005*	.001<p<.005*	.001<p<.005*	.001<p<.005*
3.) 2-5 days Rt.	p<.001*	.200<p<.300	.010<p<.020*	.010<p<.020*	.010<p<.020*	.005<p<.010*
4.) 7 days Lt.	p<.001*	.050<p<.100	.001<p<.005*	p<.001*	.005<p<.010*	.005<p<.010*
5.) 7 days Rt.	p<.001*	.600<p<.700	.050<p<.100	.100<p<.200	.900<p	.100<p<.200
6.) 2-4 wks. Lt.	.700<p<.800	.500<p<.600	.100<p<.200		.100<p<.200	.100<p<.200
7.) 2-4 wks. Rt.	.050<p<.100	.400<p<.500	.010<p<.020*		.200<p<.300	.020<p<.050*
8.) 6-7 wks. Lt.	.200<p<.300	(control)	.400<p<.500		.500<p<.600	.700<p<.800
9.) 6-7 wks. Rt.	(control)	(control)	.400<p<.500		.300<p<.400	.500<p<.600

In three cases, the catecholamine level of the experimental group was equal to that of the control group. Therefore, no p-value was entered and these three groups are considered as having returned to their control levels.

The experimental groups in which the catecholamine depletion is significant at the 0.05 level are indicated by asterisks in the preceding table. A study of this table reveals that the catecholamine values in the sham-operated animals were not significantly different from the values found in control animals. It therefore can be assumed that the operation itself does not cause any change in the catecholamine content of the heart. The decrease in catecholamine levels observed in the operated animals is believed to result directly from sympathetic denervation of the heart.

In the animals which were studied 2 to 5 days after the sympathectomy, there was a significant reduction in the catecholamine content of every portion of the heart except the left atrium. This was true regardless of the side on which the operation was performed. Both the right and the left sympathectomy caused a significant depletion of catecholamines.

The group of animals which were studied 7 days after the sympathectomy demonstrated that there was still a significant depletion of catecholamines in the right atrium.

The animals which had undergone a left sympathectomy had a significant reduction in the catecholamine content of the two ventricles and of the septum. However, in the animals in which the operation was performed on the right side, the ventricular levels were not significantly reduced from

control levels.

The animals which were studied 2 to 4 weeks after receiving a left cardiac sympathectomy had catecholamine levels which were not significantly reduced from those in control animals. In the animals studied 2 to 4 weeks after receiving a right cardiac sympathectomy, the depletion of catecholamines in the right atrium was of borderline significance, while there was a significant reduction of catecholamines in the right ventricle and in the septum. The left atrium and left ventricle were not significantly affected.

In the group of animals which were studied 6 to 7 weeks after the sympathectomy, there was no longer any significant reduction in the catecholamine level in any portion of the heart.

From a study of Table 8 as well as the catecholamine values from various portions of the heart in individual experiments as reported in the previous chapter, it can be shown that the left atrium is very seldom significantly affected by the sympathectomy. This is true for either a right or a left sympathectomy. And yet the control catecholamine level of the left atrium is not low. On the contrary, it is usually much higher than the level in either ventricle. However this level seems to remain high following a sympathectomy. The high concentration of catecholamines in atrial tissue suggests a profuse distribution of sympathetic fibers to this region of the heart. And yet sympathetic cardiac denervation seems to affect neither the content of catecholamine in the left atrium nor the functional response of the

left atrium to stellate stimulation. When the stellate ganglion is electrically stimulated, the left atrium responds with an augmentation in force of contraction which is recorded as an elevation of the A-wave in the atrial pressure recording. In the majority of the functional experiments performed on animals with a unilateral cardiac sympathectomy, the response of the left atrium to stellate stimulation is essentially the same as the response in a normal animal. At present there seems to be no obvious explanation for this apparent paradox. It would seem that removal of one-half of the sympathetic supply to the heart should significantly reduce the catecholamine content and the functional response of the left atrium. And yet it appears that removal of neither the right nor the left sympathetic supply produces this result.

Another point illustrated by the table of p-values is that removal of the left sympathetic nerve supply seems to affect the entire heart with the exception of the left atrium. However, removal of the right sympathetic supply seems to affect mainly the right atrium, the right ventricle, and the septum with a lesser effect on the left ventricle and very little or no affect on the left atrium. Consider, for example, the animals studied 2 to 5 weeks after a right sympathectomy. The catecholamine depletion in the right atrium is of borderline significance while the depletion in the right ventricle and the septum is significant. There is no significant reduction in the catecholamine level of either the left atrium or the left ventricle.

This point is further illustrated by the following four tables. Tables 9

and 10 indicate the average catecholamine values in groups of sympathectomized animals calculated as percentages of the average values for the control group.

Table 9

Catecholamine Levels Following Left Sympathectomy

	2-5 days	7 days	2-4 weeks	6-7 weeks
RA	62%	52%	105%	86%
LA	82%	62%	90%	106%
RV	55%	54%	72%	87%
LVA	64%	44%		
LVB	55%	55%	63%	90%
S	57%	54%	68%	94%

Table 10

Catecholamine Levels Following Right Sympathectomy

	2-5 days	7 days	2-4 weeks	6-7 weeks
RA	40%	48%	62%	100%
LA	74%	90%	85%	115%
RV	64%	60%	55%	124%
LVA	66%	77%		
LVB	65%	97%	80%	118%
S	61%	78%	57%	87%

If one considers the animals studied 7 days after the sympathectomy, it can be seen from Tables 9 and 10, that left sympathectomy caused a reduction in catecholamine content in all areas of the heart, the left atrium being least affected. However, a right sympathectomy caused a depletion of catecholamines mainly in the right atrium and the right ventricle.

Tables 11 and 12 give the percentage of samples in each group which showed a reduction of catecholamines to a level which was less than 2/3 of the average level of the control group.

Table 11

After a Left Sympathectomy

	2-5 days	7 days	2-4 weeks	6-7 weeks
RA	56%	86%	20%	0%
LA	22%	63%	20%	0%
RV	78%	75%	60%	0%
LVA	63%	100%		
LVB	89%	75%	60%	0%
S	67%	75%	60%	0%

Table 12

After a Right Sympathectomy

	2-5 days	7 days	2-4 weeks	6-7 weeks
RA	89%	80%	75%	25%
LA	56%	40%	25%	0%
RV	67%	60%	100%	33%
LVA	56%	40%		
LVB	56%	20%	50%	0%
S	78%	20%	75%	0%

A comparison of the effects of a left vs. a right sympathectomy shows that a left sympathectomy most often caused a reduction in the catecholamine levels of all areas of the heart except the left atrium. However a right sympathectomy most often caused a reduction in the catecholamine levels of the right atrium, the right ventricle, and the septum while it less frequently affected the left atrium and the left ventricle.

These results then lead to the conclusion that the left sympathetic nerve supply to the heart must be rather evenly distributed throughout the entire heart, since removal of this innervation seems to affect the entire heart. On the other hand the right sympathetic supply seems to serve predominantly the right side of the heart with a lesser amount going to the left side of the heart. Thus when the right supply is removed, the right side of the heart is more severely affected than the left side. As was stated before, there does not appear to be any suitable explanation for the fact that the left atrium is seldom affected by removal of either the right or the left nerve supply.

All of the tables presented so far in this chapter have clearly illustrated the fact that the catecholamine level in all areas of the heart is at its lowest value in the animals studied one week or less after the sympathectomy. In the animals studied at a longer interval after the operation, the levels tend to be somewhat higher. In fact, in the animals studied approximately 6 weeks after the sympathectomy, the catecholamine levels in the various regions of the heart are not significantly different from the corresponding

levels in the control group of animals. In an attempt to explain this increase in catecholamine levels to near normal values, it was theorized that a re-innervation of the heart may have occurred.

In the animals studied 6 weeks after the sympathectomy, the functional response of the heart also resembles the response in the normal animal. The recording obtain from animal WD 9, which was reproduced in the preceding chapter, could have come from a normal animal. Actually the animal had undergone a unilateral cardiac sympathectomy 6 weeks previously. In an attempt to explain this response, it was again theorized that perhaps the heart has been reinnervated.

It remains to be explained, however, just how this reinnervation may take place. The caudal cervical, the stellate, and the first four thoracic ganglia of the sympathetic trunk have been removed on the side of the operation. It is possible for the pre-ganglionic sympathetic fibers originating within the spinal cord to undergo regeneration after they have been cut. In all probability, this takes place in these animals after the operation. However, even though a marked regeneration of pre-ganglionic fibers may occur, this could not accomplish much toward reinnervation of the heart since the post-ganglionic cell bodies have been removed and there remain no post-ganglionic neurons with which the pre-ganglionic fibers may synapse.

A second possible means of reinnervation of the heart would be by way of post-ganglionic sympathetic neurons originating in the upper cervical or the lower thoracic (T5 and below) sympathetic ganglia which remain intact

after the operation. It may be possible for these fibers to reach the heart. However it does not seem likely that any significant reinnervation of the heart could occur by this means. And indeed, upon post-mortem examination, there was never found to be an intact nervous connection between the regenerating ends of the cut sympathetic trunk and the heart.

However, a third means exists by which reinnervation of the heart may occur. The phenomenon of collateral sprouting in post-ganglionic sympathetic neurons has been demonstrated by Murray (206). It had previously been shown that collateral sprouting occurs in pre-ganglionic sympathetic neurons and, in 1957, Murray demonstrated that this phenomenon can take place in post-ganglionic sympathetic neurons as well. Murray performed his experiments with the post-ganglionic fibers from the superior cervical ganglion. He cut 90% of the post-ganglionic sympathetic trunk, ligated the proximal and distal ends, and separated them to prevent them from rejoining. In ten weeks, there had been sufficient collateral sprouting of the remaining intact post-ganglionic fibers to restore normal function to the nictitating membrane.

Perhaps this may be taking place in the hearts under study in the present experiments. When a portion of the sympathetic post-ganglionic supply to the heart is removed, the remaining post-ganglionic fibers which originate from the opposite sympathetic trunk may undergo collateral sprouting to reinnervate the heart. This is postulated as an explanation for the fact that the catecholamine content and the functional response of

the heart begin to return to normal levels approximately 6 weeks after the operation.

In the present group of experiments, there is no direct evidence either for or against the hypothesis that collateral sprouting of post-ganglionic sympathetic fibers may be taking place. It is also not known what would constitue an adequate stimulus to the post-ganglionic fibers from the contralateral sympathetic trunk in order to initiate collateral sprouting.

In the animals which were subjected to a bilateral sympathectomy, the catecholamine levels were not reduced to zero as might at first glance be expected. In fact, as a whole, the catecholamine levels of the bilaterally sympathectomized animals were not much lower than those of the unilaterally sympathectomized animals. These results are not surprizing in the light of the data reported by Cooper in which he compared the effects of a bilateral cervico-thoracic sympathectomy with those of a complete cardiac denervation involving surgical removal of the heart followed by autotransplantation. In his study, the average left ventricular catecholamine content of control dogs was 0.71 ugm per gram of tissue with a range from 0.57 to 0.97 ugm per gram. The average catecholamine level in the left ventricle of dogs which underwent a bilateral cervico-thoracic sympathectomy was 0.49 ugm per gram of tissue with a range from 0.41 to 0.62 ugm per gram. However, in the left ventricle of dogs subjected to a complete cardiac denervation, there was less than 0.05 ugm of catecholamines per gram of tissue (54). Thus it can be seen that the bilateral sympathectomy as performed in the

present experiments is not sufficiently complete to cause a 100% depletion of catecholamine levels in the heart.

The data obtained from the control animals point to the fact that important differences in catecholamine content exist between the various regions of the heart. The average levels in the control group of animals were as follows:

RA	LA	RV	LVA	LVB	S
1.46	1.13	0.78	0.64	0.62	0.67
±0.33	± 0.40	± 0.23	± 0.25	± 0.20	± 0.23

An analysis of this data revealed that the catecholamine content of the atria was significantly higher than that of the ventricles. The difference between the combined atrial catecholamine level and the combined ventricular level was statistically significant. The p-value was less than 0.001. In every control animal, the catecholamine level was markedly higher in the atrium than in the ipsilateral ventricle. Likewise in the sham operated animals, the atrial level was in every instance higher than that of the ipsilateral ventricle. An analysis of the data from the unilaterally sympathectomized animals showed that the level in the atrium was higher than that in the ipsilateral ventricle in all but 9 out of 104 comparisons. This high concentration of catecholamines in atrial tissues is not surpirzing in view of the profound chronotropic activity of the sympathetic nerves and also the occurrence of marked augmentation in force of contraction of both atria

during stellate stimulation as evidenced by the elevation of the A-wave in the atrial pressure recording.

The concentration of catecholamines in the ventricular walls in the control animal is approximately 50% of that found in the atria, when reported as micrograms per gram of tissue. However this sympathetic innervation to the ventricles is extremely important, as evidenced by the powerful augmentation in force of contraction as a result of stellate stimulation.

In most of the hearts from the control animals, the catecholamine level was higher in the right atrium than in the left, and it was also generally higher in the right ventricle than in the left. The difference between the right and left atrial levels and between the right and left ventricular levels did not prove to be statistically significant, however; the p-value was approximately 0.10. These findings are in agreement with the data of Muscholl (208). In his study, he determined the amounts of norepinephrine and epinephrine in the various parts of the hearts of cats, rabbits, guinea pigs, and rats. In each of these species he found that the norepinephrine concentration was much higher in the right atrium than in the left and it was also higher in the right than in the left ventricle. To this data it may now be added that in the dog also there tends to be a higher catecholamine level on the right side of the heart than on the left.

One of the purposes of this investigation was to demonstrate a correlation between a catecholamine deficit as determined by the chemical analysis and a functional deficit as determined by the four-chamber pressure recordings.

It was theorized that the loss of the cardiac sympathetic transmitter as a result of the sympathectomy could be correlated with a proportional loss in cardiac function. However it was found that this correlation was often absent when individual animals were studied. In many instances, animals showing a significant catecholamine deficit gave good functional responses while animals retaining near-normal catecholamine levels gave very poor functional responses. It is evident, therefore, that no direct relationship between catecholamine content and cardiac function can be made at the present time utilizing these data.

CHAPTER V

SUMMARY

In an attempt to determine how much influence is exerted independently by the right and the left cardiac sympathetic nerves, a unilateral cardiac sympathectomy was performed. At various periods of time after the sympathectomy, the intact contralateral stellate ganglion was electrically stimulated and pressure recordings were taken from the four chambers of the heart. The content of norepinephrine and epinephrine in each of the four chambers and the septum was determined using the tri-hydroxy indole fluorimetric analysis.

The experimental results obtained were compared with data from control animals. The catecholamine content for control animals was as follows:

RA	LA	RV	LVA	LVB	S
1.46 ug/gm	1.13 ug/gm	0.78 ug/gm	0.64 ug/gm	0.62 ug/gm	0.67 ug/gm

The figures sited are the average values for a group of eight control animals. The catecholamine content of the atrium was always markedly higher than that of the ipsilateral ventricle. The difference between the combined atrial catecholamine level and the combined ventricular catecholamine level was statistically significant ($p < 0.001$). In most of the hearts from control animals, the catecholamine level was higher in the right atrium than in the left, and it was also generally higher in the right ventricle than

in the left. These differences, however, were not statistically significant.

Sham-operated animals had catecholamine levels which did not differ significantly from those of control animals. This indicates that the operation itself had no effect on the cardiac catecholamine content.

Although there is some variability among animals, the unilaterally sympathectomized animals definitely gave an abnormal response to stellate stimulation when studied within 2 weeks following the operation. In many cases the augmentation usually seen was much reduced or absent. When a full response did occur, it was frequently characterized by a considerable delay between the initiation of the stimulation and the attainment of maximum response. When functional studies were made on animals 6 or 7 weeks after the initial operation, the responses were comparable to those obtained from a normal animal. It is postulated that reinnervation of the heart accounts for this return to the normal picture.

An analysis of the catecholamine data obtained from the unilaterally sympathectomized animals showed that the most marked reduction in catecholamine levels occurred in those animals which were studied within one week after the initial operation. The catecholamine content was reduced by the operation to about 50% of the control level. By 6 weeks after the operation, however, the catecholamine content had again increased and the levels were not significantly different from the levels in the control animals.

The effect of a right sympathectomy was to reduce the catecholamine content of the right atrium, the right ventricle, and the septum, with only

a slight effect on the left ventricle and very little or no effect on the left atrium. A left sympathectomy, on the other hand, brought about a reduction in the catecholamine content of the entire heart with the exception of the left atrium. The catecholamine level of the left atrium was not significantly affected by either operation.

The fact that the animals studied 6 weeks after the initial operation demonstrate a normal catecholamine content and a normal functional response indicates that some reinnervation of the heart has occurred. For reasons stated in Chapter IV, it is postulated that this reinnervation may take place by means of collateral sprouting of the intact post-ganglionic sympathetic fibers from the contralateral side.

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APPROVAL SHEET

The dissertation submitted by Mary Ann Aberle Klouda has been read and approved by five members of the faculty of Loyola University.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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